Iron status and iron-vitamin C co-supplementation effects on cognition, subjective mood and fatigue in menstruating, non-anaemic women aged 18–49 years

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ABSTRACT

Iron is an essential nutrient required for numerous biological processes, particularly concerning brain function. However, iron deficiency is the most prevalent nutrient deficiency worldwide. Although previous research regarding the effects of iron status and iron supplementation on psychological and physiological outcomes is extensive, there are several methodological limitations relating to heterogeneity in iron status cut-offs, sample populations, and supplementation dosage, duration and formulations. Research has also largely focused on iron deficiency anaemia with less known about non-anaemic iron deficiency (NAID), despite the increased prevalence of NAID among women of reproductive age. Therefore, this thesis aimed to investigate iron status and the effects of iron bis-glycinate chelate supplementation on cognitive function, subjective fatigue, mood and wellbeing in iron sufficient (IS) and NAID women of reproductive age. Vitamin C co-supplementation was also explored as well as any differential effects of this on cognitive function, subjective fatigue, mood and wellbeing.

Chapter 2 describes a cross-sectional study that explored the association of haemoglobin and serum ferritin with cognition, subjective mood, fatigue and wellbeing whilst controlling for the confounding effects of dietary iron, menstrual blood loss, physical activity and body mass index. Chapter 3 describes a systematic review of findings from randomised controlled trials investigating the effects of iron supplementation on cognitive function, mood, fatigue and/or wellbeing in women of reproductive age. Chapters 4 and 5 describe randomised, placebo-controlled, parallel groups trials of a 16-week intervention with iron bis-glycinate chelate or iron/vitamin C co-supplementation. Effects on cognition, subjective mood, fatigue and wellbeing (Chapter 4) and cerebral haemodynamics and whole-body energy metabolism at rest and during cognitive demand (Chapter 5) were assessed in IS and NAID females.

Serum ferritin concentrations were positively associated with emotional functioning and mental health. Although iron status improved following iron/vitamin C co-supplementation, treatment effects of both iron and iron/vitamin C were limited and mixed. Iron treatment alone reduced feelings of depression and total mood disturbance yet engendered an unexpected reduction in physical component summary scores. However, iron/vitamin C treatment resulted in improved serial subtraction accuracy whilst increased total haemoglobin concentrations were found during cognitive demand for NAID women compared to IS women. Taken together, these findings suggest that iron treatment can be beneficial for certain aspects of psychological functioning and that the lack of cognitive differences between NAID and IS women of reproductive age may be due to cerebral haemodynamic compensation.

Table of Contents

LIST OF TABLES AND FIGURES

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AUTHOR'S DECLARATION

I declare that the work contained in this thesis is all my own work and has not been submitted for any other award. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The work was done in collaboration with Bayer HealthCare and analysis of biological samples collected throughout this thesis was completed internally at Northumbria University and externally at the Royal Victoria Infirmary, Newcastle-upon-Tyne.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted for all studies presented throughout this thesis by the Faculty of Health and Life Sciences Ethics Committee at Northumbria University and were conducted according to the Declaration of Helsinki (1964).

I declare that the word count of this thesis is 65,664 words.

Name: Hannah Louise Avery

Signature:

Date:

Chapter 1 **INTRODUCTION**

1.1 Background

Dietary supplements are intended to supplement the diet with vitamins and minerals when the body is not provided with sufficient levels for both exogenous and endogenous reasons such as individual diets and malabsorption, respectively. There has however been a shift in motivations behind supplement use; from prevention and treatment of diagnosed deficiencies to simply improving and maintaining overall health (Bailey, Gahche, Miller, Thomas, & Dwyer, 2013; Jenkins et al., 2018). The growing interest surrounding the association between multivitamins and better cognitive and physiological functioning (Kennedy & Haskell, 2011) and the potential of multivitamins and minerals to reduce the risk of chronic diseases (Blumberg, Bailey, Sesso, & Ulrich, 2018) has contributed to this motivational change. Consequently, dietary supplements are more frequently used by those who already have nutrient-rich diets and healthier lifestyles than those who do not use supplements (Bailey, Fulgoni, Keast, & Dwyer, 2012).

Iron deficiency (ID) is the most prevalent nutrient deficiency worldwide and is considered the most significant contributor to anaemia onset (World Health Organisation, 2008). Iron deficiency and iron deficiency anaemia (IDA) may be asymptomatic or may present with a host of symptoms including weakness and fatigue. Consequently, iron supplementation trials have been of increased interest worldwide to combat its prevalence. Such trials have largely focused upon pregnant women and infants as they are considered most at risk of developing IDA due to increased physiological and developmental needs. This IDA can occur despite iron homeostasis adaptations to meet the increased need (Fisher & Nemeth, 2017; Lönnerdal, 2017), whereas in iron-replete individuals such adaptations can enhance the vulnerability to exceeding the tolerable upper limit of iron (Brannon & Taylor, 2017). Evidence supports a U-shaped risk curve associated with iron where both low and high iron status contribute to adverse events such as low birth weight, small for gestational age, preterm delivery in response to low and high maternal iron status during pregnancy (Brannon & Taylor, 2017; Dewey & Oaks, 2017), impaired cognitive neurodevelopment (Georgieff, 2011; Lozoff et al., 2006; Lozoff & Georgieff, 2006) and cumulative high brain iron resulting in neurodegenerative disease later in life (Agrawal, Berggren, Marks, & Fox, 2017).

This introduction aims to outline the various functions of iron in the human body and the associated physiological and behavioural effects. Evaluation of iron status parameters and

several factors involved with supplementation will also be discussed, alongside dietary sources and the biochemistry of iron homeostasis.

1.2 Dietary sources and classification of iron deficiency and IDA

Iron is an essential trace element for humans as it cannot be synthesised by the body and must therefore be obtained from the diet. Fortunately, iron is in abundance in the natural environment with both plants and animals contributing to human dietary iron requirements. Average dietary iron consumption consists of approximately 5-15 mg/day of elemental iron – the total amount of iron available for absorption by the human body. This comprises haem and non-haem iron, yet the small intestine only absorbs approximately 1-2 mg/day (Zhu, Kaneshiro, & Kaunitz, 2010). Haem iron derives from the proteolytic digestion of animal sourced haemoglobin and myoglobin whereas non-haem iron is most abundant in plants such as dark green leafy vegetables and non-animal products such as breads, cereals, legumes, nuts and seeds (Saunders, Craig, Baines, & Posen, 2013). Although haem iron only constitutes 10 % of dietary iron consumed by omnivores, its tightly sequestered structure within a protoporphyrin ring ensures a greater bioavailability (20-30 % absorbed) than the less tightly sequestered non-haem form (1-10 % absorbed) (Anderson & Frazer, 2017; Beck, Conlon, Kruger, & Coad, 2014). Non-haem iron absorption is negatively affected by dietary components such as polyphenols, phytates, and tannins, but benefits from the action of small organic acids such as citric and ascorbic acid to ensure solubility and successful absorption (Anderson & Frazer, 2017).

Iron status thresholds vary dependent upon age, sex and pregnancy. For all individuals, when the body's ability to absorb iron from the diet is diminished and the demand or loss of iron is great, the body must rely on mobilisation of serum ferritin iron stores. Therefore, during this negative iron balance only serum ferritin concentrations decrease whilst all other iron status biomarkers remain stable. This is the first stage of ID and is often undiagnosed due to other normal haematologic indices. Upon depletion of these iron stores however, a progressive decrease in serum iron, transferrin saturation and hepcidin coupled with an increase in total iron-binding capacity (TIBC) and soluble transferrin receptors (sTfR) is documented (Sheikh, Hantoushzadeh, Shariat, Farahani, & Ebrahiminasab, 2017). Haemoglobin is one of the final biomarkers to be affected and respectively decrease (Figure 1.1); thresholds have been established for specified population groups to define anaemia (Table 1.1). Although haemoglobin is essential for diagnosing anaemia, the measurement of haemoglobin alone is not sufficient to diagnose the cause of anaemia as it cannot accurately reflect iron status.

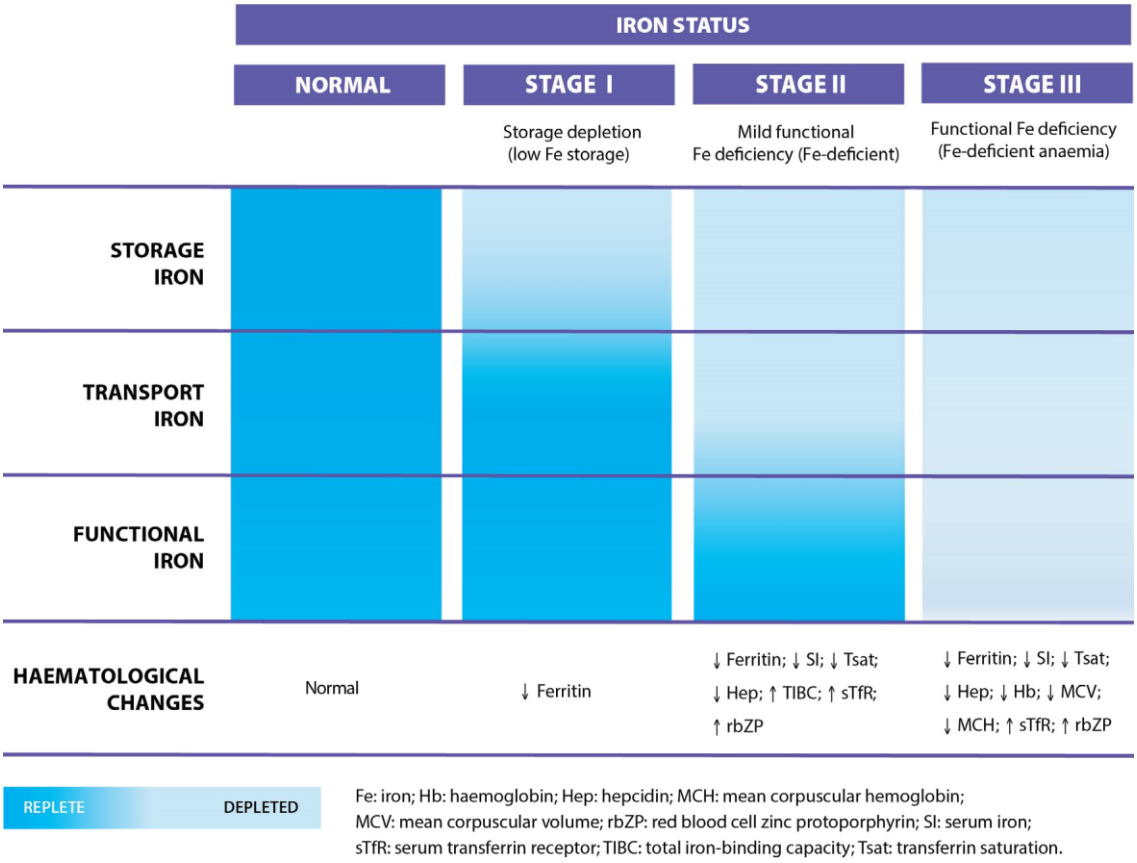


Figure 1.1 The progression of iron deficiency and associated haematological changes associated with each stage. From 'Nutritional anaemias: tools for effective prevention and control' by World Health Organisation, 2017.

Anaemia can be microcytic (insufficient haemoglobin production; IDA), normocytic (decreased blood volume and/or decreased erythropoiesis, e.g., haemoglobinopathies) or macrocytic (insufficient cell production and/or maturation, e.g., mega/non-megaloblastic anaemia) in nature; therefore, each is associated with a differential diagnosis, yet haemoglobin is reduced in all (Moreno Chulilla, Romero Colás, & Gutiérrez Martín, 2009). For an accurate assessment of iron status and diagnoses of ID and IDA at a population level, it is recommended to measure serum ferritin and sTfR alongside haemoglobin (World Health Organisation, 2007). Within intervention studies, it is recommended to use serum ferritin and haemoglobin (World Health Organisation, 2007). World guidelines currently define ID in adults, regardless of sex, as serum ferritin < 15 µg/L, and in children under 5 years of age when serum ferritin is < 12 µg/L unless presented with coexistent inflammation when ferritin is < 30 µg/L (World Health Organization, 2011b). These thresholds are to be considered alongside those for haemoglobin (Table 1.1) to determine iron sufficient (IS; haemoglobin above anaemic threshold), non-anaemic iron deficient (NAID; haemoglobin above anaemic threshold) and IDA (haemoglobin below anaemic threshold) iron status groups. As serum ferritin is also a known acute phase protein (APP), its production can be altered in response to infection or inflammation as in

normocytic anaemia of chronic disease (Nairz, Theurl, Wolf, & Weiss, 2016). Therefore, measuring markers of inflammation such as C-reactive protein (CRP) is recommended to adjust serum ferritin concentrations accordingly or to exclude individuals with elevated inflammation from controlled studies (Namaste et al., 2017).

Table 1.1 Haemoglobin thresholds used to define anaemia at sea level. Adapted from Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity by World Health Organisation, 2011

Population group	**Haemoglobin cut-off (g/L)**
Children	
➢ **Aged 6 – 59 months**	110
➢ **Aged 5 – 11 years**	115
➢ **Aged 12 – 14 years**	120
Women	
➢ **Non-pregnant women (aged ≥ 15 years)**	120
➢ **Pregnant women**	110
Men (aged ≥ 15 years)	130

1.3 Absorption and homeostasis of non-haem and haem iron

In the human body, non-haem iron can either present itself as soluble ferrous (Fe2+) or insoluble ferric (Fe3+) iron. Under physiological pHs, oxidised Fe3+ is the most stable form of iron, however this is not beneficial to iron absorption. Fe3+ may bind to transferrin to form transferrin-bound iron (TBI), which is soluble for transport into the cell. Alternatively, the acidic pH in the proximal duodenum promotes the reduction of Fe3+ to absorbable Fe2+ by the ferric reductase enzyme duodenal cytochrome B at the apical membrane of the enterocytes in the intestinal lumen (Ems & Huecker, 2020). The production of gastric acid is essential to successful iron absorption as although haem iron is absorbed independently of pH, non-haem iron requires an acidic pH to be absorbed (Tempel, Chawla, Messina, & Celiker, 2013). Fe2+ can then be absorbed into the cell by the iron importer divalent metal transporter 1 (DMT1) (Figure 1.2). Although haem iron is absorbed with greater efficacy, there is still disparity as to the exact mechanism responsible. Haem iron passes directly across enterocyte brush borders through haem-specific receptor mediated endocytosis (Silva & Faustino, 2015). It is postulated that intestinal haem is transported into the endocyte cytosol by haem carrier protein 1

(Shayeghi et al., 2005) or haem responsive gene 1 (Rajagopal et al., 2008) and is then metabolised by haem oxygenase 1 to produce Fe2+ (Jian Wang & Pantopoulos, 2011). However, in approximately one of 200-250 people, HFE gene mutations in the autosomal recessive disorder hereditary haemochromatosis (HH) lead to excess absorption of non-transferrin-bound iron by cardiomyocytes, hepatocytes and pancreatic islet cells (Golfeyz, Lewis, & Weisberg, 2018). This state of iron overload causes symptoms including abdominal and joint pain, fatigue, diabetes and heart failure (Golfeyz et al., 2018).

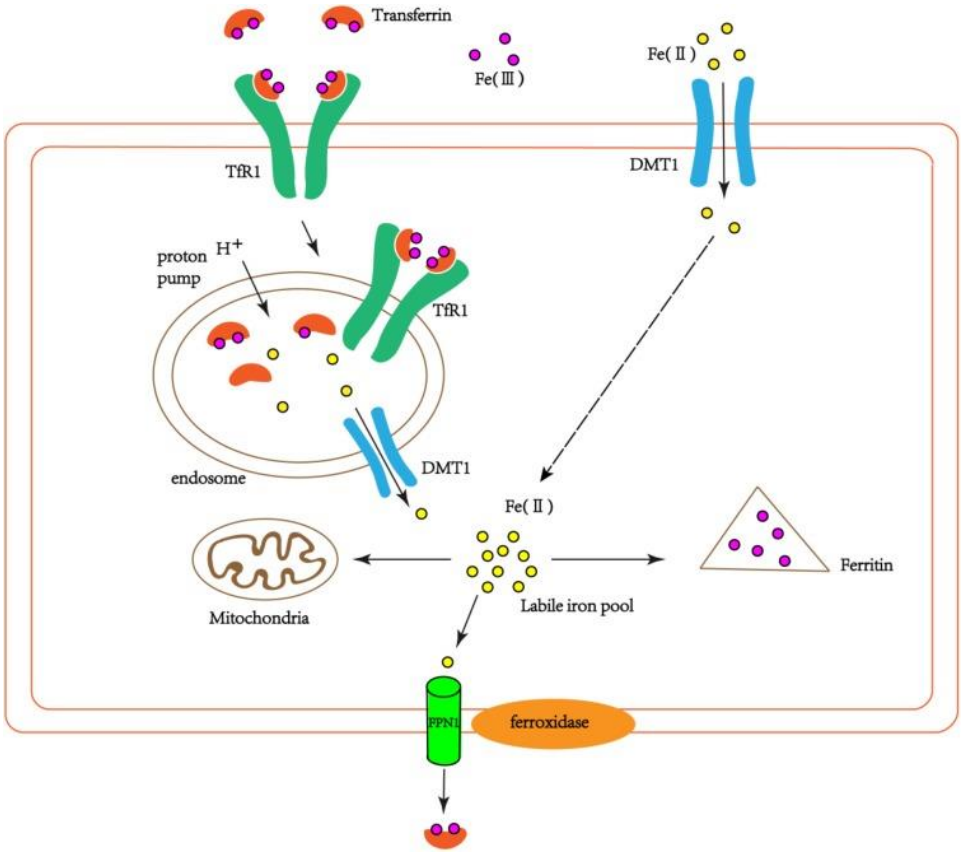


Figure 1.2 Schematic diagram of iron transport into the cells. Fe3+ can be reduced to absorbable Fe2+ in the duodenum to facilitate direct transfer into cells through DMT1. Extracellular Fe3+ may chelate plasma transferrin to form soluble transferrin bound iron (TBI) to facilitate its transport into cells. TBI binds to transferrin receptor 1 (TFR1) on the precursor cell surface initiating endocytosis of the TBI/TFR1 complex. The acidic environment of the endosome disassociates and reduces Fe3+ to Fe2+, which is transported by DMT1 into the cytosolic labile iron pool. Fe2+ may then be destined for storage within ferritin, exported out of the erythroid cells by ferroportin and oxidised to Fe3+ by ferroxidase, or for metabolic utilisation within mitochondria (Liu, Fan, Yang, Wang, & Guo, 2018).

In the cytoplasm of the enterocyte, Fe2+ ions may be sequestered within the iron-storage protein ferritin as Fe3+ within a ferrihydrite structure if iron is not immediately required elsewhere (Ems & Huecker, 2020). If iron is required outside of the enterocyte, ferroxidase activation of ferritin monomers mobilises Fe2+ ions out of the ferrihydrite structure, across the

basolateral membrane and through the transmembrane protein ferroportin into circulation to bind transferrin (Ems & Huecker, 2020; Silva & Faustino, 2015). Transferrin bound iron is mostly utilised by erythrocyte precursors in the bone marrow to support the daily production of approximately 200 billion red blood cells (RBCs) (Knutson, 2017). In humans, RBCs remain in the circulation for approximately 120 days before reaching senescence and their subsequent removal by circulating macrophages (de Back, Kostova, van Kraaij, van den Berg, & van Bruggen, 2014). Senescent RBC haemoglobin is degraded to haem and to prevent its intracellular cytotoxicity it is then degraded to Fe2+ (Chiabrando, Vinchi, Fiorito, Mercurio, & Tolosano, 2014; Gottlieb, Truman, Cohen, Leichtmann-Bardoogo, & Meyron-Holtz, 2012). This may be stored as ferritin or released into the circulation to be recycled in the process outlined in Figure 1.3.

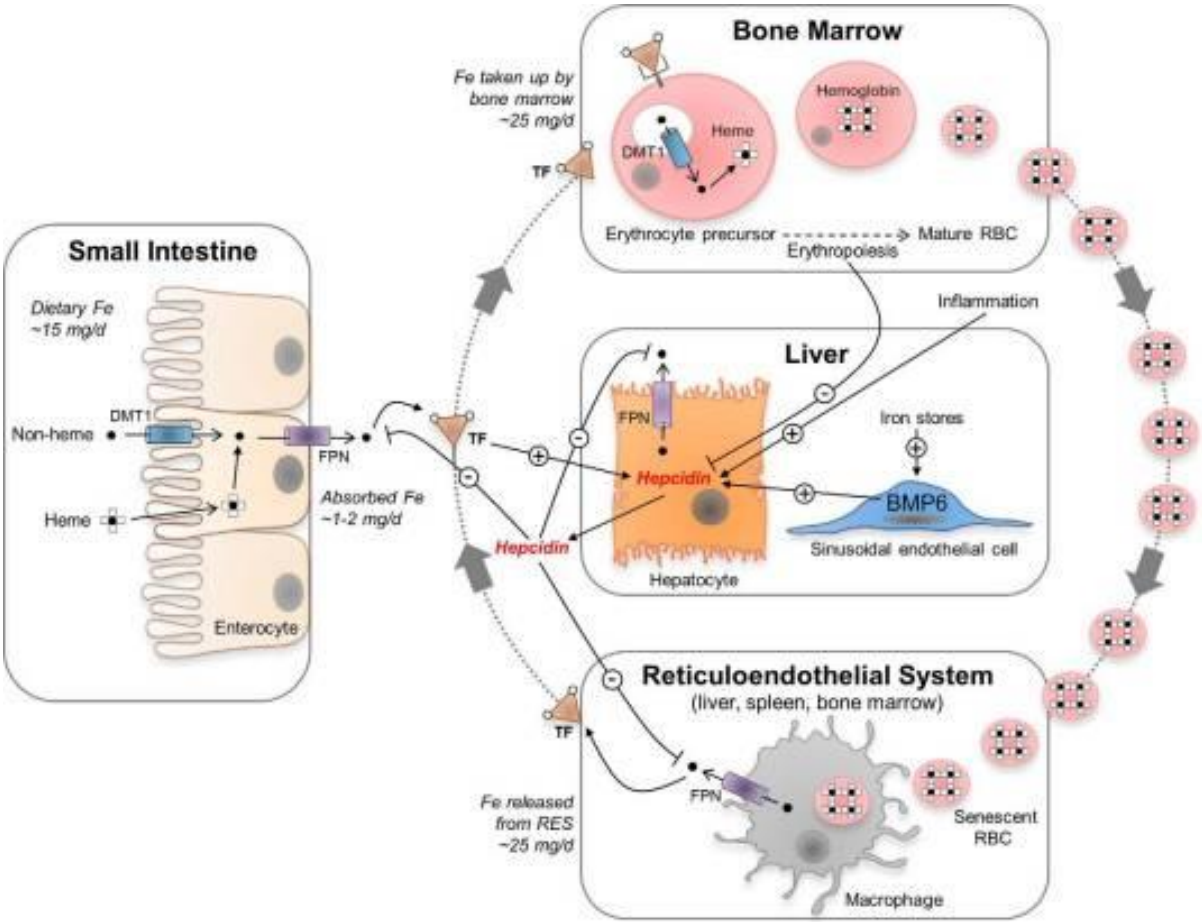


Figure 1.3 Schematic of the main cells involved in systemic iron homeostasis. Fe2+ is imported into erythroid mitochondria to synthesise haem. This is exported out of the mitochondria for incorporation into haemoproteins such as haemoglobin in newly synthesised red blood cells. Senescent or damaged RBCs are removed from the circulation macrophages of the liver, spleen and bone marrow, by erythrophagocytosis. Macrophages endocytose the senescent RBCs into the phagosome, which fuse with lysosomes forming erythrophagolysosomes, where RBCs and its haemoglobin are degraded to haem. Haem oxygenase 1 degrades cytosolic haem into Fe2+, which may be stored in ferritin or exported out of the macrophage by ferroportin to be returned to the bone marrow as TBI for reincorporation into erythrocyte precursors for further haem synthesis. Hepcidin is produced by hepatocytes to regulate iron absorption by binding to ferroportin to induce its internalisation and degradation. Liver sinusoidal endothelial cells detect fluctuations in iron stores and augment BMP6 expression, which activates the BMP-SMAD pathway for hepcidin activation (Knutson, 2017)

Iron levels in the human body are tightly controlled by the circulating peptide hormone hepcidin, produced by hepatocytes, which is up or downregulated by the liver's ability to detect intra- and extracellular iron. Hepcidin binds to ferroportin expressed on enterocytes, macrophages and hepatocytes to induce its internalisation and degradation (Nemeth et al., 2004) forcing Fe2+ ions into ferritin stores rather than into the circulating plasma (Ems & Huecker, 2020). Therefore, when iron stores are low hepcidin is downregulated, potentiating

an increase in ferroportin on the cell surface for greater Fe2+ ions efflux into the circulation. Liver sinusoidal endothelial cells detect fluctuations in iron stores and augment BMP6 (bone morphogenetic protein 6) expression, which activates the BMP-SMAD pathway for hepcidin activation (Rausa et al., 2015; Silvestri, Nai, Dulja, & Pagani, 2019). This pathway is also required in states of inflammation during which iron level fluctuations no longer drive hepcidin production; interleukin-6, an inflammatory cytokine released by macrophages during inflammation, stimulates hepcidin production and ferroportin downregulation (D'Angelo, 2013).

1.4 The functions of iron in the body

As described in section 1.3, iron is tightly regulated within the human body to ensure appropriate intra- and extracellular concentrations. It is reasonable to suggest that when homeostasis is disturbed, knock-on effects occur across cellular functioning. Iron deficiency and IDA may be asymptomatic or may comprise non-haematological symptoms including weakness, fatigue, mood changes, behavioural disturbances, an impaired immune response, reduced exercise endurance and impaired cognitive function (Beard, 2001; Beard & Connor, 2003). Similarly, iron accumulation is considered a hallmark of many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, multiple system atrophy and Huntington's disease (Ndayisaba, Kaindlstorfer, & Wenning, 2019). Such detrimental effects are believed to be a result of iron dyshomeostasis and the essential role iron must play as a modulator of various cellular processes in tissues.

1.4.1 Iron modulates the immune response

As iron is an essential mineral for human health, it is unsurprising that it is a vital component in various metabolic processes in the human body but also every living organism. However, despite its abundance in the environment, iron is often considered a growth limiting factor due to its existence as an insoluble ferric oxide (Abbaspour, Hurrell, & Kelishadi, 2014). Consequently, microbes, yeasts and fungi have adopted various mechanisms to capture iron in its most suitable form for biological function; for example, bacteria synthesise highly specific Fe3+ complexing siderophores to scavenge iron (Ward et al., 2011); yeasts and pathogenic fungi use siderophore synthesis and transport for non-reductive iron uptake alongside mechanisms of reducing ferric iron to ferrous iron (Bairwa, Hee Jung, & Kronstad, 2017). Similarly, the human body recognises the importance of metal ions for such pathogenic proliferation and subsequently has evolved various sophisticated strategies to combat bacterial invasion by reducing available iron for bacterial propagation. The recycling of iron,

as shown in Figure 1.3 is important for conserving a suitable amount of iron for the needs of the human body without excess availability for invading pathogens. Indeed, many of the genes and proteins that are involved in iron homeostasis as discussed above also have related immunological functions to infection and inflammation (Johnson & Wessling-Resnick, 2012; Porto & De Sousa, 2007). The two central players involved in the sequestration of iron for immune function are hepcidin and ferroportin. In response to an immune attack by an extracellular pathogen such as *Escherichia coli*, hepcidin expression is upregulated by IL-6 to bind ferroportin to induce the internalisation and lysosomal degradation of the complex (Nemeth et al., 2004). Iron is thereby retained within enterocytes and macrophages resulting in hypoferremia and normal to elevated ferritin preventing invading extracellular pathogens from thriving off otherwise circulating iron. In contrast, intracellular pathogens like *Salmonella* and *Legionella* promote the downregulation of ferroportin in favour of the need to retain iron within the macrophage for its own use (Paradkar et al., 2008; Willemetz et al., 2017). However, in response to such pathogens, hepcidin expression is again upregulated to induce the degradation of ferroportin, though this effect is outweighed by a larger induction of ferroportin transcription (Manfred Nairz, Haschka, Demetz, & Weiss, 2014). Subsequently, iron export is increased, and intracellular iron depleted to prevent intracellular pathogen proliferation.

Macrophages also secrete the iron-binding glycoproteins lactoferrin and lipocalin-2 for the modulation of extracellular iron in response to pro-inflammatory cytokines. Lactoferrin can sequester free iron to disrupt pathogen propagation (Legrand & Mazurier, 2010). The protein has demonstrated antimicrobial activity with its ability to enhance phagocytosis, inhibit biofilms, modify microbe-host interactions, and induce apoptosis (Siqueiros-Cendón et al., 2014). Lipocalin-2, however, directly binds to certain siderophores in their iron-laden or iron-free forms; for example, enterobactin secreted by *E. coli* and *Salmonella* and mycobactin secreted by *Mycobacterium tuberculosis* (Berger et al., 2006) (Johnson et al., 2010). Lipocalin-2 knockout mice are thus shown to have an increased sensitivity to both extracellular and intracellular pathogenic infection due to its antibacterial function and its role in maintaining proper immune functionality through modulating normal macrophage function and neutrophil maturation (Guglani et al., 2012; Wang et al., 2019).

By consequence of immune activation affecting iron distribution, it is often difficult to distinguish between absolute ID and functional iron homeostasis disturbances. Additionally, both absolute and functional deficiency may coexist, leading to challenges in interpreting iron status as serum ferritin is elevated under inflammatory conditions. Absolute ID and overload can however negatively affect immune function. Experimental evidence indicates a fundamental role of iron in normal development of the immune system due to its essential role

in immune cell proliferation (Hassan et al., 2016). The cell-mediated immune response is affected by ID as there is a decrease in T-lymphocyte numbers and increased dysfunction leading to significantly lower concentrations of pro-inflammatory cytokines like IL-6 (Aly, Fayed, Ismail, & Abdel Hakeem, 2018; Attia, Essa, Nosair, Amin, & El-Agamy, 2009; Beard, 2001; Ekiz, Agaoglu, Karakas, Gurel, & Yalcin, 2005). Iron is also necessary for monocyte and macrophage differentiation (Collins, 2003) and acts as a cofactor in the oxidative burst mechanism for eliminating bacterial invasion (Slauch, 2011); thus, during ID, such bactericidal activity is attenuated. However, the effects of ID upon humoral immunity remain controversial; it is suggested to be less affected in comparison to its cell-mediated counterpart (Beard, 2001; Kuvibidila, Kitchens, & Baliga, 1999), yet evidence in children and pregnant women also suggests decreased immunoglobulin G (IgG) concentrations (Ekiz et al., 2005; Feng, Yang, & Shen, 1994; Hassan et al., 2016; Tang et al., 2006). However, in non-pregnant women of reproductive age with IDA, serum immunoglobulins were not significantly lower than controls (Sadeghian et al., 2010). In iron overload it is instead postulated that both the cell-mediated and humoral immune responses are altered as T-cell numbers are reduced, affecting B-cell function (Walker & Walker, 2000). A dysregulated immune response is shown to affect social behaviours and promote neuropsychiatric disorders such as depression, schizophrenia and autism spectrum disorders (Gibney & Drexhage, 2013; Takahashi, Flanigan, McEwen, & Russo, 2018). Animal and human studies have also indicated a role for immune cells in cognitive function. Depletion of the cell-mediated immune response in adult mice significantly impairs spatial learning and memory (Kipnis, Cohen, Cardon, Ziv, & Schwartz, 2004), as does transfusing immune cells from older mice into younger mice (Brynskikh, Warren, Zhu, & Kipnis, 2008). Respectively, transferring immune cells or blood from young mice can reverse the impairments in cognitive function (Villeda et al., 2011; Villeda et al., 2014). In healthy older adults, lower numbers of CD4+ T cells are associated with better cognitive performance, as they are the major pro-inflammatory cytokine producer among T cells (Serre-Miranda et al., 2015) and are associated with reduced hippocampal neurogenesis (Wolf et al., 2009). It is postulated that chronic inflammation-stimulated microglial activation induces the amplification of additional pro-inflammatory cytokines and reactive oxygen species (ROS) causing enhanced neuronal iron uptake (Wang, Song, Jiang, Wang, & Xie, 2013). Iron accumulates in a region-specific manner in the ageing brain; increased microglial iron content and the deposition of insoluble protein aggregates that colocalise iron are associated with neurodegeneration in Alzheimer's disease and Parkinson's disease (Ndayisaba et al., 2019). Overall, iron plays a key role in pathogenic survival, however the human body has developed efficient mechanisms to overcome such attacks. Yet when iron status is compromised, tipping the optimal level to a deficient or overload status, immunological function may also be compromised to allow increased proliferation of infectious organisms and induce deleterious

effects on brain function and behaviour. Similarly, reduced immune activation can preserve cognitive function in the ageing brain.

1.4.2 Iron modulates DNA metabolism and mitochondrial function

One of the mechanisms postulated to account for the detrimental effect of ID on the immune system is iron-sensitive DNA metabolism. Iron-sulphur clusters function as cofactors for enzymes important for successful DNA synthesis and repair including replicative DNA polymerases and primases, DNA helicases, nucleases, glycosylases and demethylases (Puig, Ramos-Alonso, Romero, & Martinez-Pastor, 2017). Ribonucleotide reductase (RNR) similarly is an iron-dependent enzyme responsible for the catalysis of the rate-limiting step in the *de novo* synthesis of deoxyribonucleoside triphosphates (dNTPs) necessary for DNA synthesis, replication and repair (Sanvisens, Bano, Huang, & Puig, 2011). Iron deficiency decreases the amount of iron available for its cofactor role, though the mechanisms used to adapt towards such a deficiency to continue successful DNA metabolism require further investigation. In iron deficient states, yeasts and bacteria are shown to replace iron-dependent cellular processes with iron-independent alternatives (Sergi Puig, Vergara, & Thiele, 2008) or by reducing the cellular demand for iron, respectively (Masse, Salvail, Desnoyers, & Arguin, 2007). Such mechanisms are not possible in mammalian systems due to the requirement of iron as a cofactor (Sanvisens et al., 2011); although, there is evidence to suggest potential strategies for preferential optimisation of RNR over other iron-using processes as its activity is either stable or increased when ID is in its early stages (Furukawa, Naitoh, Kohno, Tokunaga, & Taketani, 1992).

Iron deficiency and iron overload increase oxidative stress through mitochondrial dysfunction. Iron deficiency anaemia is associated with a greater degree of DNA damage in comparison to healthy controls and this significantly correlates with the severity of IDA and oxidative stress (Aslan et al., 2006). Excess iron accumulation can be cytotoxic, generating increased ROS to induce DNA damage, which may eventually lead to cell apoptosis (Nakamura, Naguro, & Ichijo, 2019). Iron deficiency and overload disrupt iron-sulphur cluster formation and mediation of electron transfer in the respiratory electron transport chain for adenosine triphosphate (ATP) generation (Paul, Manz, Torti, & Torti, 2017). Mitochondrial damage also infers a reduction in haem for oxygen transport, decreased respiratory control and decreased gluconeogenesis (Klempa, Willis, Chengson, Dallman, & Brooks, 1989; Masini et al., 1994); dysfunction of these cellular functions is known to be a direct cause of moderate to severe fatigue (Nicolson, 2014). This is bolstered by experimental evidence suggesting that iron deficiency provokes a hypoxic response in human cardiomyocytes causing mitochondrial dysfunction, diminished ATP

production and impaired contractility by consequence of reduced iron-sulphur complex activity (Hoes et al., 2018). Energy depletion because of ID is also shown to affect dendritic mitochondrial motility in the hippocampus during neuronal development (Bastian, von Hohenberg, Georgieff, & Lanier, 2019). Such evidence may be relevant to cognitive and behavioural impairments and brain functions in neurodegenerative and neuropsychiatric diseases inflicted by changes in brain iron homeostasis, mitochondrial functioning and subsequent energy metabolism (Manji et al., 2012; Streck et al., 2014; Wu, Chen, & Jiang, 2019)

1.4.3 Iron modulates oxygen transport

In humans, myoglobin and haemoglobin are the primary oxygen-binding metalloproteins. Both contain iron but function differently; myoglobin is an oxygen storage protein providing oxygen to muscle tissue whereas haemoglobin is involved in oxygen transport from the lungs to peripheral tissues (Corey, Kimmel, & Leonard, 2014). The primary species of normal adult haemoglobin is haemoglobin A, which accounts for approximately 92 % of all total haemoglobin concentrations (Pittman, 2011). Haemoglobin is a tetramer, consisting of four globin chains; two alpha chains and two beta chains (a2b2) each with a haem group located in crevices near the exterior of the molecule that bind oxygen to create oxyhaemoglobin (Figure 1.4).

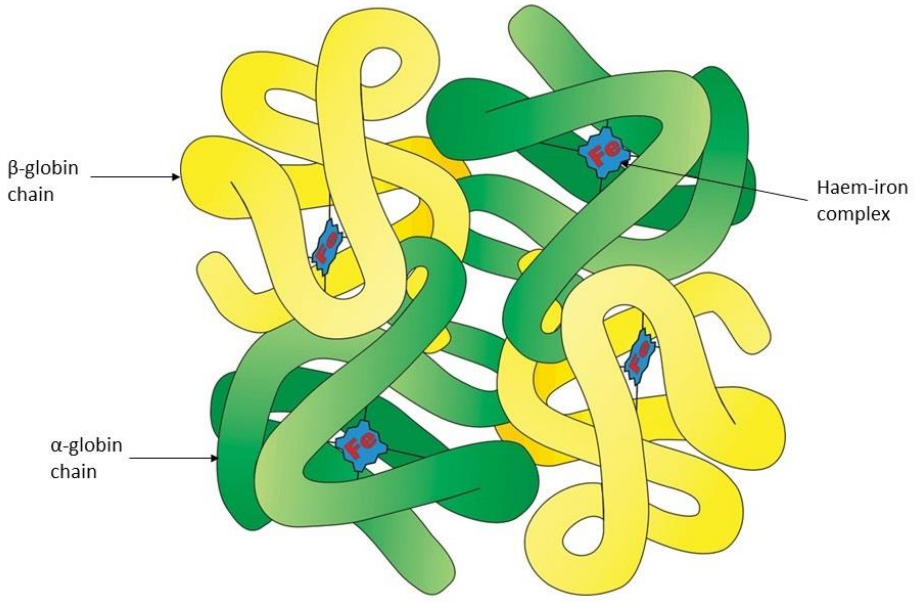


Figure 1.4 Schematic of human haemoglobin A molecule; two α-globin chains (green), two β-globin chains (yellow), haem moiety consisting of a protoporphyrin ring and a ferrous iron ion. Adapted from Thomas and Lumb (2012).

Each haemoglobin molecule can therefore simultaneously bind up to four oxygen molecules. Haemoglobin exists in a taut form (T) and a relaxed form (R). Haemoglobin has allosteric properties, thus as oxygenation ensues the globin chains alter their shape to the R-form, increasing oxygen affinity for the remaining haem groups (Figure 1.5).

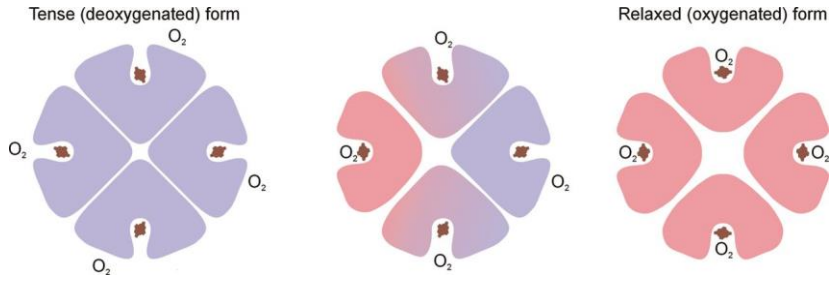


Figure 1.5 The allosteric properties of haemoglobin initiating structural changes from 'tense' to 'relaxed'. In the deoxygenated form, access to the haem molecule is restricted due to the tense structure. When oxygen does bind the haem molecule, a structural change is initiated in the haemoglobin protoporphyrin ring. Interactions between adjacent globin chains are relaxed to allow easier access by oxygen to the haem molecule (Thomas & Lumb, 2012).

This quaternary conformation change is facilitated by an alkaline pH that prompts the dissociation of hydrogen bonds and salt bridges, thereby improving oxygen affinity and inducing haemoglobin oxygen saturation (Thomas & Lumb, 2012). This can occur in the alveoli under high partial pressures and within a high pH environment, yet under acidic conditions, such as in the peripheral tissues, the T-form of haemoglobin is favoured. Hydrogen bonds and salt bridges are formed between the globin chains ensuring a low affinity for oxygen and instead favouring protons, carbon dioxide and organic phosphates, promoting the release of oxygen in a process known as the Bohr effect (Dajnowicz et al., 2016). Therefore, when the quantity of functional haemoglobin is reduced, anaemic hypoxia may present due to a diminished oxygen-carrying capacity of erythrocytes. Consequently, the heart adapts by increasing cardiac workload through increases in blood volume, preload, heart rate and stroke load, whilst decreasing afterload to deliver an adequate amount of oxygen to the tissues, including the brain (Pereira & Sarnak, 2003). This adaptation potentiates a lesser capacity for exercise and the occurrence of dyspnoea and fatigue; prolonged and exacerbated IDA can cause compromised cardiac function and result in cardiomyopathy or heart failure (Hegde, Rich, & Gayomali, 2006). Similarly, increasing cardiac workload has been shown to invoke an increase in cerebral blood flow as a neuroprotective compensatory mechanism against IDA to enhance cerebral oxygen delivery (Aliefendioglu et al., 2007; Hare, 2004). Neurovascular coupling may explain this mechanism as an optimal blood supply to the brain is required for the metabolic consumption of oxygen and glucose as neural fuel substrates (Phillips, Chan, Zheng, Krassioukov, & Ainslie, 2016). It therefore seems rational for a compound that can

modulate oxygen transport and cardiovascular function to have secondary effects upon brain function.

1.4.4 Iron modulates myelination

Oligodendrocytes are iron-containing glial cells in the central nervous system and are the principal cells in the brain that stain for iron, which is consistent with knowledge of iron levels being greater in myelin-rich white matter than in grey matter (Connor & Menzies, 1996). Oligodendrocytes are responsible for myelination of axons, which is required for enabling rapid saltatory nerve conduction and axon integrity (Nave & Werner, 2014). Human myelin comprises 70 % lipids, 27.7 % of which is cholesterol (Brady, Siegel, Albers, & Price, 2005) and iron is a cofactor required for the biosynthesis of each. Similarly, the role of iron in oxidative metabolism indirectly affects myelinogenesis. In the developing brain, iron-containing oligodendrocytes colocalise with the myelinogenic foci; this functional relationship between myelin production and iron accumulation is consistent with the observation that the highest acquisition of iron in the central nervous system coincides with peak myelination (Connor & Menzies, 1996). Iron uptake at peak myelination is associated with its essential role in oxidative metabolism (Todorich, Pasquini, Garcia, Paez, & Connor, 2009). Of all cells in the brain, oxidative metabolic activity is highest in oligodendrocytes; this is maintained by iron-requiring enzymes (i.e., glucose-6-phosphate dehydrogenase) (Cammer, Snyder, Zimmerman, Farooq, & Norton, 1982). The pentose-phosphate pathway for glucose metabolism is used by oligodendrocytes and reportedly 60 % of glucose used by the brain during peak myelination is metabolised via this pathway (Connor & Menzies, 1996). Following maximal rates of myelination, the amount of glucose utilised by the pentose phosphate pathway reduces to 25 % and iron consumption is reduced despite no decrease in iron staining or utilisation (Cammer, 1984; Todorich et al., 2009). As iron is clearly involved in optimal oligodendrocyte function, ID can lead to hypomyelination during brain development. Animal and human studies have provided support for this (Algarín, Peirano, Garrido, Pizarro, & Lozoff, 2003; Ortiz et al., 2004; Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998; Yu, Steinkirchner, Rao, & Larkin, 1986), and there is increasing evidence to suggest that oligodendrocytes continue to require a steady iron supply to maintain a consistent metabolic rate. Restricting dietary iron in the postweaning period (postnatal days 21-63) of rats significantly reduces markers of myelination and oligodendrocyte metabolic activity in the cerebrum and hindbrain (Beard, Wiesinger, & Connor, 2003). This suggests that beyond early brain development, postnatal ID can affect the production and maintenance of myelin as oligodendrocytes require a consistent supply of iron to meet their metabolic needs. Myelin deficits in humans reduce information processing speed across the nervous system, which is

essential for higher-order cognitive functions, including attention, learning, memory, planning, moderating social behaviours and regulating mood (Nickel & Gu, 2018). This implies an essential role of iron across the lifespan for myelin facilitation of whole brain neuroplasticity, brain function, behaviours and mood.

1.4.5 Iron modulates neurotransmission

Brain maturation extends from childhood and adolescence through to adulthood and includes changes in neurotransmitter systems; the development and maturity of the monoaminergic neurotransmitter systems exhibit a higher sensitivity spanning childhood and adolescence (Pitzer, 2019). During this time period, concentrations of monoamine transporters and receptors are continuously modified, initially playing important roles in axonal growth and synapse formation and switching to the role of neurotransmission with age (Beard, 2003). Altered levels of neurotransmitters are associated with several health conditions. Dopaminergic and noradrenergic systems' dysfunction has been associated with attention deficit hyperactivity disorder (ADHD) (Konofal et al., 2008) and schizophrenia (Insel, Schaefer, McKeague, Susser, & Brown, 2008); coupled with the white matter changes associated with lipid and cholesterol synthesis dysfunction causing profound myelin and oligodendrocyte abnormalities (Davis et al., 2003). Additionally, dysfunction of serotonergic, noradrenergic and/or dopaminergic systems underlies the monoamine hypothesis of the pathophysiology of depression (Delgado, 2000), whilst increased anxiety is also associated with dysfunction of serotonergic and noradrenergic systems as a consequence of altered hippocampal brain-derived neurotrophic factor (BDNF) signalling (Texel et al., 2012).

The synthesis of monoamines serotonin, dopamine and noradrenaline begins with amino acid tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (dopamine and noradrenaline). Both enzymes are non-haem iron-dependent monooxygenases responsible for the catalysis of the iron-mediated incorporation of molecular oxygen into the amino acid substrate and the reducing substrate tetrahydrobiopterin to give the hydroxylated products L-DOPA and 5-hydroxytryptophan, which are converted into dopamine (and subsequently noradrenaline) and serotonin respectively (Roberts & Fitzpatrick, 2013). Certainly, it is to be expected that as iron modulates monoamine synthesis through its co-factor role, that a reduction in iron for this iron-dependent neurotransmitter synthesis pathway would result in a decline in neurotransmitter production and activity. Although, it is argued that brain ID rarely causes a reduction in the activity of these enzymes (Youdim, Ben-Shachar, & Yehuda, 1989) suggesting a method of iron conservation for efficient enzyme function in iron deficient states. Recent evidence suggests that in those with ID, lowered serum tryptophan concentrations are available for the

rate-limiting initial step in serotonin synthesis (Wenninger et al., 2019), which may subsequently contribute to the development of mood disorders.

Changes in brain iron levels due to ID have demonstrated a relationship with impaired emotional behaviours through altered monoaminergic metabolism. Iron deficiency has a universally negative impact upon monoamine production but specific to brain region and stage of neural development (Kim & Wessling-Resnick, 2014). Severe ID in children and young adults has been shown to affect dopamine frontal-striatal circuits and subsequently neurocognitive and motor functioning (Algarin et al., 2013; Lozoff, 2011). Animal studies have also identified down-regulation of dopamine receptors 1 (D1) and 2 (D2) in the striatum and prefrontal cortex in ID (Erikson, Jones, Hess, Zhang, & Beard, 2001). D2 regulates dopamine clearance via dopamine transporter (DAT), where the activity and density in an ID state can be downregulated to increase striatal dopamine (Meiergerd, Patterson, & Schenk, 1993). Extracellular dopamine is also elevated in the caudate putamen and nucleus accumbens due to decreased DAT density (Nelson, Erikson, Pinero, & Beard, 1997) yet is reduced in in the prefrontal cortex in comparison to IS controls, which is suggested to be echoed by increased anxiety (Li et al., 2011). By consequence of dopamine dysregulation, newly diagnosed IDA patients aged 45 years and above have demonstrated a greater risk of developing Parkinson's Disease four or more years after the initial anaemia diagnosis (Hong et al., 2016). Low serum ferritin and increased serum hepcidin have also been associated with ADHD (Wang, Huang, Zhang, Qu, & Mu, 2017; Yazici, Yazici, & Ustundag, 2019).

Concentrations of extracellular noradrenaline are also elevated by consequence of ID. Dopamine-β-hydroxylase activity is increased by 75 % in caudate homogenates of iron deficient rats in comparison to controls, increasing noradrenaline concentrations (Bianco, Wiesinger, Earley, Jones, & Beard, 2008). This hydroxylase would normally produce noradrenaline from dopamine; thus, its elevation is postulated to be a compensatory mechanism for brain ID and the altered dopamine response (Bianco et al., 2008). Results are however conflicting regarding the impact ID has on serotonin concentrations. Rat studies have shown both reductions (Shukla, Agarwal, Chansuria, & Taneja, 1989) and increases in serotonin (Mackler, Person, Miller, Inamdar, & Finch, 1978) following ID, whilst no significant differences in prefrontal cortex concentrations in comparison to controls have been shown (Li et al., 2011). However, it is interesting to note that the prevalence of IDA is more frequent amongst psychiatric patients in comparison to the general population (Korkmaz et al., 2015), whilst low serum ferritin and haemoglobin concentrations have been associated with both depressive and anxiety disorder incidence (Lever-van Milligen, Vogelzangs, Smit, & Penninx,

2014; Vahdat Shariatpanaahi, Vahdat Shariatpanaahi, Moshtaaghi, Shahbaazi, & Abadi, 2007).

Beyond the monoaminergic system, ID can disrupt the balance between inhibitory and excitatory neurotransmitters, γ-aminobutyric acid (GABA) and glutamate, respectively (Ferreira, Neves, & Gozzelino, 2019). Iron deficiency causes elevation of GABA in the hippocampus; this is suggestive of an increased inhibitory drive for reducing neurotransmission and subsequent brain activity when energy is limited (Rao, Tkac, Townsend, Gruetter, & Georgieff, 2003). The excitatory role of glutamate is subsequently dampened by ID due to a decrease in glutamate decarboxylase and glutamate dehydrogenase (Mittal, Pandey, Mittal, & Agarwal, 2003). Animal models have identified ID-associated elevations in glutamate in prenatal and postnatal models, suggesting an increased synthesis or a decreased release from the neurons (Rao et al., 2003). However, glutamatergic neurotransmission utilises >80 % of the total energy expenditure in the brain (Attwell & Laughlin, 2001) and it is therefore postulated that glutamatergic neurotransmission is decreased in ID because of inefficient energy metabolism (Rao et al., 2003). Coupled with a decrease in glutamate catalytic enzymes, and a reduction in glutamate binding synaptic membranes (Agarwal, 2001), this leads to high intracellular glutamate levels yet an attenuation of glutamatergic signalling in ID. Together, these findings suggest that neurotransmitter pathways are influenced by iron status, which can have detrimental effects upon neural activity inducing changes in cognition and mood.

1.4.6 Summary of functions

There is a large body of evidence to suggest the essential role of iron in a plethora of fundamental cellular functions amongst living organisms. To ensure its longevity, the human body has developed protective mechanisms to control iron levels to combat its central role in pathogen propagation. Additionally, iron acts as an essential cofactor for DNA metabolism and mitochondrial function, resulting in optimum ATP generation for cellular processes. The essential role of iron in haemoglobin and myoglobin synthesis is important for oxygen transport to the tissues and muscles. Furthermore, there is evidence to suggest an abundant requirement of iron in the brain for optimal oligodendrocyte function throughout the lifespan and for the development and maintenance of neurotransmitter systems. Disruption of optimal iron levels is shown to impair these fundamental biological parameters and diminish brain function, behaviour, mood and fatigue.

1.5 The behavioural effects of iron

Due to the essential role held by iron in the human body, numerous observational and randomised controlled trials (RCTs) have been conducted to explore the impact of iron and iron status on brain function, behaviour, mood and fatigue. As stated previously, infants and pregnant women are most at risk of ID; however, non-pregnant women of reproductive age are also at risk due to suboptimal iron consumption and menstrual blood losses, with >20 % experiencing ID during their lives (Harvey et al., 2005; Percy, Mansour, & Fraser, 2017). Consequently, the majority of iron supplementation research focusses upon infants and pregnant women due to the clinical nature and the previous notion that ID can only affect brain functioning during neurodevelopment (Beard, 1995; Hermoso et al., 2011). However, the brain's plastic nature allows it to adapt both structurally and functionally beyond infancy, highlighting that an iron insult can detrimentally affect neural functioning at any time of life (Oberman & Pascual-Leone, 2013). The following sections will therefore review the current literature surrounding the behavioural effects of iron in infants, older children, pregnant women and non-pregnant women of reproductive age of varying iron status.

1.5.1 The role of iron in mood

Iron deficiency has previously been associated with mood disorders such as depression and anxiety. Depression is considered an affective disorder that is characterised by negative feelings affecting daily functioning; over 264 million people of all ages suffer from depression and it is currently the leading cause of disability worldwide (James et al., 2018), with incidence twice as common in women than in men (Ma, Xu, Wang, & Li, 2019). A possible mechanism responsible for this involves the serotonergic system. The serotonin receptor 5-HT_{1A} modulates the release of serotonin to be cleared by serotonin transporters (SERT). Positron emission tomography scans of healthy women indicate a higher density of 5-HT_{1A} receptors and a lower density of SERT in comparison to healthy men (Jovanovic et al., 2008). This is also demonstrated in those with depression as SERT are significantly decreased in depressed women whereas no significant differences are demonstrated between depressed and healthy men (Staley et al., 2006). As women are more at risk for ID than men, this highlights a potential role of iron in the aetiology of depression as IDA is associated with increased reports of depressive symptoms in women (Pamuk et al., 2015; Vahdat Shariatpanaahi et al., 2007), and to a lesser extent in men (Hidese, Saito, Asano, & Kunugi, 2018; Yi et al., 2011). Animal models have shown lower densities of SERTs in the striatum of iron deficient mice (Morse, Beard, Azar, & Jones, 1999), which may be responsible for a decreased serotonin uptake by brain synaptic vesicles that can however be corrected following iron supplementation

(Kaladhar & Narasinga Rao, 1982). Similarly, dysfunction of the dopaminergic system in ID increases extracellular dopamine and downregulates functions of the DAT and D2 receptor in the striatum (Erikson, Jones, & Beard, 2000), deficits of which predict reductions in striatal functional connectivity in depression (Hamilton et al., 2018).

The prescription of antidepressants has more than doubled over a period of 13 years (Ilyas & Moncrieff, 2012), which has largely been driven by an increase in selective serotonin reuptake inhibitors (SSRIs) (Mars et al., 2017). Considerable efforts to clarify the role of iron in depression's pathophysiology through dietary interventions have been made, given that nutritional interventions are considered inexpensive, safe, easier to administer and are generally accepted by patients in comparison to antidepressants (Bodnar & Wisner, 2005). In 2017, the first nutritional guidelines to curb depression were published recommending a Mediterranean diet, rich in omega-3 fatty acids and avoiding processed foods (Opie et al., 2017) following suggestions for nutritional medicine as a mainstream treatment in psychiatry (Sarris et al., 2015). Subsequently, a Mediterranean dietary intervention was associated with positive changes in depressive symptoms in adults with major depression (Opie, O'Neil, Jacka, Pizzinga, & Itsiopoulos, 2018). This has encouraged the development of a nutrient profiling system for depression; analysis revealed iron to be one of the 12 antidepressant nutrients related to the prevention and treatment of depression (LaChance & Ramsey, 2018).

Antidepressants are considered the first-line treatment for women with moderate-to-severe postpartum depression (PPD) (Kim, Epperson, Weiss, & Wisner, 2014). Post-partum depression is a non-psychotic depressive episode that typically arises due to hormonal fluctuations following childbirth and the psychological adjustment to motherhood, affecting approximately 10-15 % of mothers annually (Anokye, Acheampong, Budu-Ainooson, Obeng, & Akwasi, 2018). There is evidence to suggest associations between PPD development and having ID with or without anaemia (Albacar et al., 2011; Corwin, Murray-Kolb, & Beard, 2003; Etebary, Nikseresht, Sadeghipour, & Zarrindast, 2010; Milman, 2011). A review of evidence linking anaemia and/or low ferritin to PPD revealed a higher risk of PPD in anaemic women and in those with low ferritin in the postpartum period rather than during pregnancy (Wassef, Nguyen, & St-Andre, 2019). By consequence, a decreased risk of PPD following postpartum iron supplementation was found, yet if iron supplementation was provided during pregnancy no protective effects were observed (Wassef et al., 2019). Potential mechanisms for an increased risk of PPD in postpartum women are reflected by changes in monoamine neurotransmitter concentrations compared to healthy controls; plasma and serum serotonin concentrations are significantly lower in postpartum women whilst noradrenaline is significantly higher (Xie, Xie, Krewski, & He, 2018; Yildiz et al., 2017). However, plasma and

serum concentrations may not reflect serotonin and noradrenaline levels in the brain. Further studies are required to elucidate the association between the risk of PPD in anaemic and iron deficient women during the postpartum period; however, a clear association between decreased iron levels and PPD presentation is evident. As women naturally suffer from detriments to the monoaminergic system in comparison to men, this may be exacerbated in a synergistic relationship between depressive and ID conditions; with evidence suggesting that PPD symptoms (depression, anxiety and stress) can be improved following iron supplementation (Beard et al., 2005; Sheikh et al., 2017; Wassef et al., 2019).

Iron deficiency during pregnancy can be detrimental to foetal well-being. Low maternal iron stores are replicated in neonates due to a limited iron-acquisition capacity of the foetus to obtain a sufficient iron supply (Jaime-Perez, Herrera-Garza, & Gomez-Almaguer, 2005). Evidence suggests low serum ferritin concentrations can continue through to early childhood (Hay et al., 2007) with increased vulnerability to anaemia during the first year of life (Scholl, 2011). Iron deficiency with and without anaemia is associated with increased fearfulness as a sign of anxious behaviours in infants, regardless of iron therapy administration (Lozoff, Brittenham, Viteri, & Urrutia, 1982). This has also been reflected in animal models with iron deficient rats demonstrating increased fearfulness and anxious behaviours in light/dark box and maze studies (Beard, Erikson, & Jones, 2002; Li et al., 2011). It is now of increasing concern that these mood alterations can persist into adulthood, despite prompt iron repletion during infancy (Kennedy, Wallin, Tran, & Georgieff, 2016). There is evidence to suggest that adolescents treated for severe, chronic ID in infancy were of more concern to parents and teachers through reports of increased symptoms of depression, anxiety, social and attentional problems (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000). These mood deficits are also evident at a follow-up of 25 years from diagnosis, with poorer emotional health increasing negative emotions and feelings of dissociation and detachment via behavioural problems observed in adolescence (Lozoff et al., 2013). Concurrently, the diagnosis of IDA during childhood or adolescence increases the risk of mood disorders including anxiety and depression alongside a developmental delay, autism spectrum disorder and ADHD (Chen et al., 2013). The explanation behind iron deficits in infancy affecting adolescent and adult mood and behaviour is no doubt complex. However, a potential mechanism to account for these later differences emerges from the detrimental effects of ID upon the nervous system during neurodevelopment. As discussed in section 1.4, ID can impair myelination, neurotransmitter function and alter neurometabolism in areas of the brain such as the prefrontal cortex, striatum and hippocampus that undergo considerable maturation in the neonatal period. When such effects occur during neurodevelopment, it is postulated that they can elicit a cascading effect, disrupting fundamental neural processes and inducing the divergence of developmental

trajectories away from the norm (Lozoff et al., 2013). For example, the hippocampus is highly metabolically active in the early postnatal period and IDA animal models have demonstrated altered neural metabolic activity that persists into adulthood regardless of iron repletion (Carlson, Stead, Neal, Petryk, & Georgieff, 2007). This is reflected by differences in brain connectivity in adulthood. In comparison to healthy controls, former IDA young adults have decreased connectivity of the left posterior cingulate cortex (PCC) with posterior default mode network (DMN) regions (Algarin et al., 2017). This pattern of functional connectivity is also observed in major depressive disorder (Bluhm et al., 2009; Yang et al., 2016), Alzheimer's disease (Chhatwal et al., 2013), ADHD (Castellanos et al., 2008) and social functioning in schizophrenia (Dodell-Feder, Delisi, & Hooker, 2014). Therefore, it would be reasonable to suggest that an iron-related insult during the neonatal period and infancy has potentially irreversible neurological effects regardless of blood and brain iron repletion later in life (Georgieff, 2011).

Due to the overwhelming attention on PPD, maternal anaemia and the potential consequences upon infant neurodevelopment, minimal research has focussed upon the role of iron in mood regulation in non-pregnant women. Meta-analytic findings indicate an inverse association between dietary iron intake and risk of depression, however only amongst Asian populations of both genders (Li, Li, Song, & Zhang, 2017). Studies that have investigated women of reproductive age specifically have produced conflicting results. Two cross-sectional studies found that a higher frequency of depressive symptoms was only associated with low serum ferritin when women of reproductive age were also taking oral contraceptives (Fordy & Benton, 1994; Rangan, Blight, & Binns, 1998). However, current evidence suggests a lack of negative associations between oral contraceptives and depression (Toffol, Heikinheimo, Koponen, Luoto, & Partonen, 2012), although female adolescents may be more susceptible (Skovlund, Mørch, Kessing, & Lidegaard, 2016). Recent evidence suggests that the combined presentation of depressive symptoms and oral contraceptive use is only reported amongst 16-year-old girls, which may impede quality of life and increase the risk of contraceptive non-adherence (de Wit et al., 2020). Therefore, when disregarding oral contraceptive use and those < 20 years of age, it may be suggested that no observational relationship between iron haematological indices and depression exists in women of reproductive age. However, participants were not screened for inflammatory and blood disorders, the use of iron supplements or medications that could affect iron status biomarkers, all of which could inflate serum ferritin and inaccurately depict iron status (Namaste et al., 2017). When such factors are considered, the frequency of NAID is shown to be 15 % higher in female medical students classed as depressed (Vahdat Shariatpanaahi et al., 2007). To infer a role of iron in the aetiology of depression, iron supplementation RCTs measuring depression change scores

have been conducted. However, no associations have been found between improvements in iron status and depressive symptoms following oral iron supplementation over four (Verdon et al., 2003) or 12 weeks (Vaucher, Druais, Waldvogel, & Favrat, 2012). The main outcome of both studies was to assess the effect of iron supplementation upon fatigue and specifically recruited participants reporting considerable fatigue without obvious clinical causes. Subsequently, depression was assessed as a secondary outcome, giving rise to the suggestion that more comprehensive depressive scales may have yielded different results (Lomagno et al., 2014). As a decrease in anxiety levels was however observed following four weeks of iron supplementation (Verdon et al., 2003), this highlights the potential benefits of iron to non-pregnant women for improving indices of mood.

Although complex, there is adequate evidence to suggest a role for iron in mood regulation amongst infants, children, adolescents, pregnant and non-pregnant women. However, despite non-pregnant women presenting with an increased risk of mood disorders and ID in comparison to men, a causal relationship between the two has yet to be established. This demographic is deserving of further cross-sectional studies and RCTs using non-clinical samples and validated, comprehensive mood scales to determine the potential mood enhancing effects of iron and to establish whether normalcy can be restored following iron repletion.

1.5.2 The role of iron in fatigue and physical performance

Reports of fatigue in general practice have consistently shown that women are more likely to report to their general practitioner for fatigue than men (Cullen, Kearney, & Bury, 2002; Engberg, Segerstedt, Waller, Wennberg, & Eliasson, 2017; Ridsdale et al., 1993). There is evidence to suggest that ID is associated with a higher prevalence of constant tiredness in comparison to those who are IS (Patterson, Brown, Powers, & Roberts, 2000). The role of iron in oxygen transport, detailed in section 1.4.3, is often postulated as a cause of fatigue symptoms due to the reduced delivery of oxygen to the tissues requiring it by consequence of a reduction in haemoglobin concentrations. However, this would only be the case when haemoglobin is reduced in IDA, whereas reductions in serum ferritin in NAID should not affect the oxygen-carrying capacity of erythrocytes directly. Studies have instead suggested that reduced ferritin in NAID rats is detrimental to mitochondrial oxidative enzyme activity, such as cytochrome C, reducing successful oxygen extraction and utilisation from haemoglobin, halting the production of ATP for energy metabolism and increasing ROS production to promote oxidative stress (Bahadir, Erduran, Değer, Birinci, & Ayar, 2018; Srinivasan & Avadhani, 2012). This provides further support for the U-shaped risk curve as iron overload is

also associated with increased ROS, which is detrimental to cellular vitality. To combat this, a change of fuel selection for skeletal muscle energy metabolism is postulated in order to limit processes with a high iron expenditure. A shift towards anaerobic metabolism is proposed as evidence suggests greater lactate dehydrogenase activity, skeletal muscle fibre alterations and a subsequent shift from oxidative metabolism to glycolysis in iron deficient rats in comparison to healthy controls (Henderson, Dallman, & Brooks, 1986; Ohlendieck, 2013). However, glycolysis is inferior to mitochondrial oxidative metabolism as only two ATP molecules are generated per glucose molecule compared to the 36 produced by oxidative metabolism (Yetkin-Arik et al., 2019). This therefore provides a mechanism for a reduced aerobic capacity in ID with and without anaemia.

There is evidence to suggest that ID is more prevalent amongst athletes in comparison to more sedentary individuals, which has the potential to affect athletic performance due to impaired aerobic capacity and increased fatigue (Sinclair & Hinton, 2005). Female athletes are at an additional risk for ID with and without anaemia (Koehler et al., 2012; Sinclair & Hinton, 2005), with an estimated prevalence of five to seven times higher than their male counterparts (DellaValle & Haas, 2011). Additionally, female athletes are at a heightened risk of NAID compared with female non-athletes (27 % vs. 13 %) (Ponorac et al., 2019). Explanations beyond those previously mentioned for women of reproductive age include increased blood loss through haematuria and the gastrointestinal tract; and intravascular haemolysis as a result of exercise intensity and the type of exercise (i.e., foot strike in runners, tissue pressure in strength training) (Beard & Tobin, 2000; Mairbäurl, 2013; McClung, Gaffney-Stomberg, & Lee, 2014). An increased erythrocyte turnover is shown in animal and human studies. Exercise-trained male rats with ID exhibit a higher erythrocyte turnover in comparison to non-exercise trained rats (Tobin & Beard, 1989). This turnover is approximately 20 % faster in human female athletes than in non-athletes; regardless of exercise training category, erythrocyte turnover is also faster than in adult men (Ehn, Carlmark, & Hoglund, 1980). Despite this, an increased erythrocyte and haemoglobin concentration in comparison to sedentary individuals is observed (Mairbäurl, 2013). Female athletes also exhibit changes in iron absorption in response to inflammation. Skeletal muscles act as secretary organs during physical activity; responding to exercise by secreting pro-inflammatory myokines such as IL-6 in an exponential fashion, which return to baseline concentrations following exercise cessation (Hojman et al., 2019). Acute exercise-induced inflammation upregulates expression of hepcidin, affecting efficient iron absorption in the gastrointestinal tract and enterocyte and macrophage iron export (McClung et al., 2014). This detrimentally affects iron status as there are inadequate amounts of iron to support physical functioning. A review of evidence to determine the effects of acute exercise on elevations in hepcidin demonstrated that a single

session of endurance exercise at a moderate-vigorous intensity augments hepcidin levels between 0 hours and 6 hours, with a peak concentration at approximately 3 hours post-exercise (Domínguez et al., 2018). It is suggested that this timing is likely to coincide with an athlete's meal consumption and so their dietary-derived iron intake is not as efficiently absorbed potentially explaining the prevalence of iron deficiency amongst this population (Peeling, 2010). However, it is suggested that iron status facilitates this hepcidin upregulation as serum ferritin < 30 µg/L is shown to blunt exercise-induced hepcidin (Peeling et al., 2014), and this is shown to increase following iron supplementation (Burden, Pollock, et al., 2015). Consequently, those considered at a sub-optimal iron status with sufficient haemoglobin concentrations and serum ferritin 30-50 µg/L may be most affected by exercise-induced hepcidin elevation due to the greater potential for hepcidin to suppress efficient iron absorption and utility, thus increasing the likelihood for ID presentation. Methods of increasing iron stores through dietary or supplementation means therefore often target female athletes to prevent ID-associated declines in sporting performance.

A meta-analytic review of 17 published RCTs investigating the effects of iron supplementation on iron status and aerobic capacity of NAID endurance athletes found that iron treatment significantly improved serum ferritin, serum iron, transferrin saturation and haemoglobin in both male and female athletes (Burden, Morton, Richards, Whyte, & Pedlar, 2015). These effects were not only larger in female groups but were also present up to eight weeks following oral treatment cessation. Iron supplementation was also seen to moderately improve maximum oxygen capacity ($VO_{2\ max}$) in NAID female endurance athletes, which is in agreement with a further meta-analysis focussing on women of reproductive age regardless of iron status and athleticism (Pasricha, Low, Thompson, Farrell, & De-Regil, 2014). However, further meta-analytic reviews have found equivocal results that do not support iron supplementation consistently improving athletic performance in NAID male and female adults (Houston et al., 2018; Rubeor, Goojha, Manning, & White, 2018). Although Houston et al. (2018) found no significant improvements in objective physical capacity amongst adults regardless of athleticism, a serum ferritin cut off of ≤ 20 µg/L and below proved effective for improving performance following iron supplementation amongst NAID athletes (Rubeor et al., 2018). Although serum ferritin concentrations for ID remain uncertain, the cut off of 20 µg/L sits between previous recommendations of < 15 µg/L and < 30 µg/L (Daru et al., 2017); reflecting the general recognition of ID from previous studies regarding female athletes often using serum ferritin cut offs between 12 and 23 µg/L (DellaValle, 2013). These findings provide support for the physical performance of trained and untrained NAID females being amenable to iron supplementation in a greater fashion than that of their male counterparts, especially at a serum ferritin threshold of ≤ 20 µg/L.

Fatigue is not only associated with a reduction in endurance aerobic capacity and physical performance. A reduction in physical parameters may present alongside central fatigue categorised by subjective feelings of lowered energy and motivation, which may instigate an increase in anxious and depressive mood depleting cognitive capacity and quality of life (Dziembowska, Kwapisz, Izdebski, & Żekanowska, 2019). The known dependency of iron in monoaminergic systems for neurotransmission, as discussed in section 1.4.5, may be critical to the presentation of fatigue-associated symptoms. The monoamine hypothesis of central fatigue suggests that an elevated ratio of serotonin to dopamine in the brain is associated with tiredness and lethargy, instigating fatigue onset, in comparison to a low ratio driving the opposite with increased arousal, motivation and feelings of reward (Davis & Bailey, 1997; Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006). As ID is associated with increases in serotonin and decreases in dopamine, the central fatigue hypothesis acts as a possible explanation for the concurrent presentation of reduced subjective energy levels and cognitive capacity. A meta-analysis of cross-sectional studies demonstrated a significant association between NAID and subjective fatigue in populations screened for RCTs and those with diagnosed disorders, but not the general population (Yokoi & Konomi, 2017).

Meta-analytic reviews have revealed significant therapeutic effects of iron supplementation in those presenting with fatigue and NAID (Houston et al., 2018; Yokoi & Konomi, 2017). Although neither review specifically focussed on women, all seven studies included across the meta-analyses investigated subjective fatigue in NAID menstruating women. Five were associated with a significant reduction in subjective measures of fatigue (Beutler, Larsh, & Gurney, 1960; Favrat et al., 2014; Krayenbuehl, Battegay, Breymann, Furrer, & Schulthess, 2011; Vaucher et al., 2012; Verdon et al., 2003). A significant response to iron supplementation was discovered in all studies when serum ferritin was ≤ 50 μg/L; a much higher cut off in comparison to studies showing iron supplementation to significantly affect aerobic capacity and physical performance. However, these studies all recruited women whose primary concern was unexplained fatigue and so a stronger association between iron and fatigue improvements is to be expected in comparison to studies where participants do not self-report fatigue or reduced physical performance. Promising findings are demonstrated amongst NAID adolescent girls; intravenous iron improved subjective fatigue and quality of life without significant improvements in haemoglobin (Sharma, Stanek, Koch, Grooms, & O'Brien, 2016). This suggests that the mechanisms underpinning the beneficial effect of iron therapy on reducing fatigue are independent of the haematological functions as suggested by the monoamine hypothesis of central fatigue. It is therefore imperative that not only objective measures of physical performance are investigated in iron supplementation RCTs, but also

subjective measures of fatigue and energy. Further studies of the general population are also warranted.

1.5.3 The role of iron in sleep quality

The key role of iron for modulation of monoamines in the brain and the role of those monoamines in sleep physiology suggests a detrimental impact of ID upon sleep parameters. Subjective sleep quality is shown to significantly predict fatigue (Lavidor, Weller, & Babkoff, 2003), quality of life (Marques, Meia-Via, da Silva, & Gomes, 2017), academic performance (El Hangouche et al., 2018; Maheshwari & Shaukat, 2019), and mood (Augner, 2011) in non-clinical populations. Regardless of the presentation of depression and anxiety symptoms, IDA is shown to affect sleep quality with IDA patients exhibiting poorer sleep quality than healthy controls (Murat et al., 2015). Dopamine neuromodulation is fundamental to sleep-wake cycle regulation, encompassing modulation of REM sleep quality, quantity and timing (Dzirasa et al., 2006; Monti & Monti, 2007; Takeuchi et al., 2018). Sleep/wake disturbances are associated with dopamine losses (Murat et al., 2015) and, as discussed in section 1.4.5, decreased brain iron is directly tied to dopaminergic system dysfunction including D1 and D2 receptor downregulation. This bolsters the idea that altered dopamine-related behaviours and dopaminergic system functioning in ID would affect sleep parameters.

Iron deficiency-associated impairments in neuronal iron uptake can trigger the pathophysiology of restless leg syndrome (RLS) (Guo et al., 2017). A significant association between IDA and RLS presentation in women has been demonstrated (Telarović & Čondić, 2019) and RLS patients have significantly reduced iron and serum ferritin concentrations in cerebrospinal fluid (Mizuno, Mihara, Miyaoka, Inagaki, & Horiguchi, 2005). This reduces available iron for dopaminergic system regulation potentially leading to the characteristic clinical feature of RLS, the irresistible urge to move the limbs whilst at rest that may only be relieved by movement. Poor sleep quality is therefore a serious consequence to RLS sufferers as a result of this (Bogan, 2006). Meta-analytic findings suggest that iron supplementation can improve RLS severity; however, subjective sleep quality did not significantly favour iron treatment (Trotti & Becker, 2019). Further RCTs are warranted to determine the impact of iron supplementation upon sleep quality.

In addition to dopamine, serotonin, noradrenaline and GABA-ergic systems are dysregulated during ID; it is postulated that these alterations affect the neural mechanisms involved in sleep organisation to cause altered REM sleep transitions observed in former IDA children despite iron repletion (Peirano et al., 2010). Effective sleep is also essential for myelin maintenance

and turnover for healthy white matter and altered sleep patterns are evident in demyelinating diseases (de Vivo & Bellesi, 2019). Iron is also essential for myelination, though iron supplementation RCTs are limited to infant and child populations for determining the effect upon sleep quality. Children with autistic spectrum disorder (ASD) are a population at risk for ID who experience altered sleep patterns, which are shown to improve following iron supplementation (Dosman et al., 2007). Similarly, sleep quality and reduced night waking significantly improved in IDA children and adolescents with sleep-maintenance insomnia treated with iron (Mila et al., 2013). Non-anaemic iron deficiency is also associated with sleep disorders such as RLS, periodic leg movement disorder and restless sleep disorder (Munzer & Felt, 2017), symptoms of which improve following iron therapy (Simakajornboon et al., 2003). Significant improvements in serum ferritin following iron supplementation are associated with sleep quality improvements in children with such conditions (DelRosso et al., 2019). As NAID is most prevalent in women of reproductive age, and there is a higher prevalence of poor sleep quality in females than males (Fatima, Doi, Najman, & Mamun, 2016; Madrid-Valero, Martínez-Selva, Ribeiro do Couto, Sánchez-Romera, & Ordoñana, 2017), further iron supplementation RCTs are warranted to determine any beneficial effects upon sleep quality in this population.

1.5.4 The role of iron in maintaining brain health across the lifespan

1.5.4.1 The role of iron in neurodevelopment and infancy

As early brain development is a highly metabolically taxing process, optimal brain development is reliant upon vital nutrients such as glucose, branched chain amino acids and iron for supporting effective cellular metabolism (Wullschleger, Loewith, & Hall, 2006). However, nutrition can be wilfully altered and may differ according to parental food habits and feeding strategies. As discussed in section 1.4, iron is essential for neurocognitive processes including neurotransmission and myelination. However, despite brain development continuing across the lifespan due to continued neural plasticity (Power & Schlaggar, 2017) it is argued that the opportunity to influence later function is far greater during early life than in adulthood (Georgieff, Brunette, & Tran, 2015). Reducing ID prevalence is therefore not only important because of its ubiquity worldwide, but also because of the potential long-term effect on neurocognitive functioning and behaviour.

Infant ID may arise as a consequence of maternal anaemia because of the high requirements for iron by both mother and foetus (Harvey et al., 2013). Premature labour also increases risk

for ID as most foetal iron is obtained from the mother during the third trimester of pregnancy, which can result in iron stores reflective of a low birth weight (Moreno-Fernandez, Ochoa, Latunde-Dada, & Diaz-Castro, 2019). Similarly, infants subject to intrauterine growth restriction and infants born to mothers with diabetes or preeclampsia are at an increased risk of developing ID (Abu-Ouf & Jan, 2015; Coşkun, Bilgen, Özdemir, Şirikçi, & Özek, 2012; Verner et al., 2007). Systematic and meta-analytic reviews have found negative associations between maternal diabetes and infant's cognitive and psychomotor development (Adane, Mishra, & Tooth, 2016; Camprubi Robles et al., 2015). There is also evidence to suggest that the children of mothers with preeclampsia and those affected by intrauterine growth restriction are at an increased risk of neurocognitive, behavioural and motor development deficits (Hartkopf et al., 2018; Korzeniewski et al., 2017; Nomura et al., 2017; Warshafsky, Pudwell, Walker, Wen, & Smith, 2016). However, such investigations have not considered the effect of consequent ID during development as a causal link for impaired cognitive and behavioural function.

Iron deficiency and IDA in infancy is associated with dysfunction of cognitive, affective and motor domains in comparison to their IS counterparts (Beltran-Navarro, Matute, Vasquez-Garibay, & Zarabozo, 2012; Carter et al., 2010). Infancy is considered a time of peak central nervous system formation of myelin, dendrites and synapses alongside hippocampal and cortical regional development (Lozoff et al., 2006). However, early life ID may encourage the prioritisation of available iron to red blood cells over the brain (Dallman, 1986). Animal models have highlighted the vulnerability of the hippocampus to early ID; a significant loss of cytochrome c oxidase activity in the hippocampus and the prefrontal cortex during foetal/neonatal ID (Bastian, von Hohenberg, Mickelson, Lanier, & Georgieff, 2016) is greatest during the early stages of dendrite maturation causing altered hippocampal dendritic growth (Bastian et al., 2016; de Deungria et al., 2000) that may persist from infancy through to puberty (Fretham, Carlson, & Georgieff, 2011). Such abnormalities may contribute to the memory and learning deficits attributed to early ID. Children born with low cord serum ferritin and chronic ID are shown to have lower scholastic performance (Tamura et al., 2002), frontostriatal-mediated executive functions and recognition memory (Geng et al., 2015; Lukowski et al., 2010), working memory and immediate and delayed recall memory (Riggins, Miller, Bauer, Georgieff, & Nelson, 2009). When provided with support, memory task deficits were improved indicating that alternate learning approaches may be enlisted by formerly ID children.

Similarly, impaired myelination is a potential mechanism for motor deficits in early life as the corticospinal tract, responsible for signal communication between the motor and sensory regions of the cerebral cortex and the spinal cord, is not fully myelinated until aged 20-40

months (Parazzini, Baldoli, Scotti, & Triulzi, 2002). As myelin is also necessary for communication with sensory cortical regions, it is vital for effective auditory and visual system functioning critical to social interaction and learning. Auditory brainstem responses (ABR) and visual evoked potential (VEP) latencies decrease through infancy to adulthood reflecting maturation of the CNS and increased myelination of the auditory and optic nerves (Algarín et al., 2003; Long, Wan, Roberts, & Corfas, 2018). Iron deficient anaemic infants however show longer ABR latencies relative to IS controls indicating slower auditory pathway transmission (Roncagliolo et al., 1998). However, NAID animal models have demonstrated a different mechanism; instead of myelination deficits, altered ABRs were a consequence of disrupted axonal maturation and development of the auditory nerve (Lee, Strathmann, Gelein, Walton, & Mayer-Pröschel, 2012). This is reflected in electrophysiological evidence that highlights the inability of ID infants to discriminate a novel from a familiar stimulus in the same manner as IS infants (Deregnier, Nelson, Thomas, Wewerka, & Georgieff, 2000; Siddappa et al., 2004). Iron is therefore key to successful auditory and visual systems, however not only during infancy. A follow-up study demonstrated consistently longer ABR and VEP latencies in formerly IDA children relative to IS controls (Algarín et al., 2003), providing support for a long-term effect of infant ID later in life.

The long-term effects of ID during infancy are also reflected in cognitive function. Children diagnosed with severe IDA as infants demonstrated poorer intelligence and visual-motor performance at 5 years (Lozoff, Jimenez, & Wolf, 1991). Ten years following ID treatment, the same children attained significantly poorer arithmetic achievement, written expression, motor functioning, selective recall, spatial memory and were more likely to have either repeated a school year or to have received additional tutoring (Lozoff et al., 2000). Cognitive and motor functioning alterations were also coupled with affective domain changes in early adolescence; a greater prevalence of anxiety and depression symptoms were evident as well as externalising behavioural problems (Lozoff et al., 2000). In addition to iron-dependent CNS developmental processes previously discussed, affective and motor functioning alterations are consistent with changes in iron-dependent neurotransmitters such as dopamine and serotonin (section 1.4.5). When these children were followed-up to the age of 19 years, cognitive test scores remained significantly lower for those who had ID in infancy relative to those who were IS (Lozoff, Jimenez, & Smith, 2006). When combined with socio-economic status (SES), those with ID in lower-SES families were doubly burdened; initial cognitive scores in infancy were 10 points lower, which extended to 25 points by 19 years old (Lozoff et al., 2006). As ID disrupts and delays neurodevelopment, it is suggested to subsequently alter infant-caregiver interactions that may further compromise development (Lozoff et al., 2006). Therefore, when combined with a lower-SES background, where there are functionally

significant differences regarding educational attainment and future career opportunities, it seems reasonable to suggest ID-associated deficits would be exacerbated. When SES was controlled for at 25 years follow-up, a greater percentage of those who had chronic ID in infancy failed to complete secondary school or pursue further education in comparison to those who were IS via the indirect path of poorer cognitive functioning in early adolescence (Lozoff et al., 2013).

Iron supplementation during infancy for prevention of ID in at risk populations has demonstrated benefits upon behaviour (Berglund et al., 2018; Berglund, Westrup, Hagglof, Hernell, & Domellof, 2013; Lozoff, Castillo, Clark, Smith, & Sturza, 2014), and psychomotor development (Angulo-Barroso et al., 2016; Chmielewska et al., 2019; Friel et al., 2003; Lind et al., 2004; Moffatt, Longstaffe, Besant, & Dureski, 1994; Stoltzfus et al., 2001). However, cognitive benefits from RCTs supplementing iron are much less pronounced. Benefits to information processing speed are demonstrated in well-nourished children (Lozoff et al., 2003) but have more recently been contradicted with no effects on neurodevelopment of well-nourished children shown (Iglesias Vázquez et al., 2019). Overall neurodevelopmental benefits of iron supplementation are quashed by systematic and meta-analytic reviews failing to identify a clear benefit of iron supplementation on cognitive function in infancy following short-term ID prevention or treatment (Pasricha, Hayes, Kalumba, & Biggs, 2013; Sachdev, Gera, & Nestel, 2005; Szajewska, Ruszczynski, & Chmielewska, 2010; Thompson, Biggs, & Pasricha, 2013; Wang, Zhan, Gong, & Lee, 2013). Such disparity has also been shown following the long-term effects of iron supplementation on cognitive performance. Following ID correction during infancy, cognitive performance was similar at 5 years of age to those who were IS at baseline (Lozoff et al., 1991). However, regardless of initial iron status, iron supplementation administered in infancy did not lead to long-term cognitive performance improvements at 7-9 years of age (Pongcharoen et al., 2011), with one study discovering a worsened performance on tasks of executive function and fine motor function when compared with those who did not receive iron supplementation from 12-18 months of age (Murray-Kolb et al., 2012). This comparatively inferior cognitive performance was also seen at a follow-up of 10 (Lozoff, Castillo, Clark, & Smith, 2012) and 16 years of age (Gahagan, Delker, Blanco, Burrows, & Lozoff, 2019) following treatment with iron-fortified or low-iron formula. Additionally, both studies found that infants with high haemoglobin (> 128 g/L) displayed poorer cognitive and visual-motor integration if they had received an iron-fortified formula compared with those who were randomised to the low-iron formula, and those with a lower baseline haemoglobin (<105 g/L) demonstrated better cognitive and visual-motor outcomes. Animal models provide possible explanations for poorer neurodevelopmental outcomes; excess iron during infancy is postulated to be neurotoxic and thus detrimental for brain

development (Berggren et al., 2016; Fredriksson, Schröder, Eriksson, Izquierdo, & Archer, 1999; Hare et al., 2015). The pro-oxidative consequences of excess iron are suggested to augment brain oxidative stress to decrease serotonin and dopamine whilst increasing the production of ROS and hydroxyl radicals following brain cell damage producing significant cognitive deficits (Yu, Feng, Shen, & Li, 2011).

Together, the evidence regarding human and animal models supports the idea of long-term, irreversible neuropsychological alterations following early iron deficiency (Beard et al., 2006; Georgieff, 2011). Further research is suggested due to the heterogeneous nature of the populations, intervention duration, dosage and cognitive tests enlisted by previous RCTs (Larson, Phiri, & Pasricha, 2017). However, there are potential implications for an optimal amount of iron for supplementation and that supplementation during infancy should be individualised based on baseline iron measures to avoid the potentially detrimental effects of early iron exposure on brain development.

1.5.4.2 The role of iron in children and adolescents

Although pre-school infants and young children are at a greater risk of ID, global statistics indicate that approximately 25 % of older children (5-9 years) and adolescents (10-19 years) are affected by IDA (Global Burden of Disease Pediatrics et al., 2016). A systematic review demonstrated significant associations between poor cognitive, behavioural and psychomotor outcomes in infants, children and adolescents born to women who were ID and IDA during pregnancy (Iglesias, Canals, & Arija, 2018). Iron deficient anaemic children aged >2 years old are also shown to have cognitive function deficits and poorer scholastic achievements in comparison to IS children (Grantham-McGregor & Ani, 2001). Whilst IDA children demonstrate the poorest cognitive function, a dose-response relationship has been postulated between haemoglobin and cognitive function in NAID primary-school-aged children (average age 9.6 years); language and arithmetic scores increased with haemoglobin concentrations, whilst this relationship was not shown in IS children (Sungthong, Mo-suwan, & Chongsuvivatwong, 2002). More recent studies have also suggested primary-school-aged children with NAID to have poorer processing speeds and visual motor-coordination in comparison to healthy controls (Hamid Jan, Amal, Rohani, & Norimah, 2010). Optimising cognitive abilities in primary-school-aged children is shown to enhance cognitive capacities in later life (Ritchie, Bates, Der, Starr, & Deary, 2013) and it may therefore be imperative to investigate optimal iron status and supplementation methods beyond infancy and into childhood.

Iron supplementation significantly improved verbal and nonverbal learning and memory when children aged 6-11 years were IDA at baseline but not when NAID (Baumgartner et al., 2012). This is in line with evidence suggesting that IDA children are most likely to benefit from supplementation (Grantham-McGregor & Baker-Henningham, 2010). Systematic and meta-analytic reviews support this as children >7 years of age responded to iron supplementation with an improved intelligence quotient (IQ) (Sachdev et al., 2005), as well as improved cognitive domains of attention and concentration (Falkingham et al., 2010), but only amongst those who were IDA at baseline. Similarly, systematic evidence of iron supplementation amongst primary school children aged 5-12 years indicates improved measures of attention, concentration and global cognition in comparison to placebo (Low, Farrell, Biggs, & Pasricha, 2013). However, global cognition improvements were only exhibited for children who were IDA at baseline, as were improvements in IQ.

Adolescence is usually associated with an increase in dietary intake to fulfil the energy requirements of accelerated growth and production of bone and muscle. Iron requirements dramatically increase to satisfy the haemoglobin demand for expansion of total blood volume, myoglobin for increases in lean body mass and the enzymes necessary for growth in both boys and girls (Beard, 2000; Mesias, Seiquer, & Navarro, 2013). Serum ferritin stores are anticipated to be redistributed for haemoglobin production, which increases the likelihood of NAID. This may be especially apparent amongst young males due to a greater incorporation of iron into erythrocytes to compensate for greater growth rates in comparison to young females (Mesias et al., 2013). However, menarche onset instigates an increase in iron requirements, and it is suggested that girls with menses are 2.57 times more likely to be iron deficient compared to girls prior to menarche onset across countries of varied economic stature (Moschonis et al., 2013). Menarche onset averages around 12.9 years of age, dependent upon ethnicity (Campbell, Mallappa, Wisniewski, & Silovsky, 2013) and menstruation for greater than three years is considered a risk factor for NAID (Sekhar, Murray-Kolb, Kunselman, Weisman, & Paul, 2017). This coincides with a time of cognitively demanding school years and as iron plays a key role in neurodevelopment, its deficiency may impair and alter the typical neurodevelopmental trajectory associated with adolescence. Significant positive associations between serum ferritin, haemoglobin and academic achievement have been shown; IDA adolescent girls demonstrated poorer academic achievement in comparison to their IS peers (Soleimani & Abbaszadeh, 2011). Such findings have also been extended to IDA and NAID adolescent girls exhibiting poorer scholastic performance through lower arithmetic, IQ, verbal learning, attention, concentration, verbal memory and recognition scores as compared to their IS equivalents (Devaki, Chandra, & Geisser, 2009; More, Shivkumar, Gangane, & Shende, 2013; Mousa, Higazi, Saleh, & Ali,

2016). As has consistently been shown in younger children, IDA adolescent girls had the lowest cognitive scores whilst those who were IS performed best with NAID girls at an intermediate level between the two. Iron deficient anaemic adolescent girls are 1.73 times more likely than non-anaemic girls to have impaired cognitive function (Bahrami et al., 2019).

A meta-analysis of iron supplementation in adolescents and young adults found evidence to support its use for improving measures of attention and concentration independent of baseline iron status, however further well-designed studies were called for (Falkingham et al., 2010). No effect on memory was found, however this may be due to the studies included not having useable data for the meta-analysis. Early iron intervention studies in pregnant adolescent girls from a low SES background identified improvements in short-term memory following iron supplementation of one month (Groner, Holtzman, Charney, & Mellits, 1986). The triple burden of adolescence, pregnancy and low SES increases the risk for ID, however ID prevalence at baseline was minimal. This high-risk demographic benefitted from iron supplementation regardless of baseline iron status, which highlights the potential for beneficial effects of iron interventions even when iron status is considered optimal. However, iron requirements during pregnancy increase from .08 mg/day in the first trimester to > 6 mg in the third (Bothwell, 2000). As this study recruited females during the first 16 weeks' gestation, it may not accurately reflect the efficacy of iron supplementation for cognitive performance enhancement in pregnancy. Similar findings are demonstrated when recruiting non-pregnant adolescents based upon iron status parameters; NAID adolescent girls verbal learning and working memory improved following iron supplementation (Bruner, Joffe, Duggan, Casella, & Brandt, 1996; Lambert, Knaggs, Scragg, & Schaaf, 2002) as did short-term memory, long-term memory and intelligence amongst NAID and IDA adolescent girls and boys (Devaki et al., 2009). Significant baseline differences existed with better cognitive performance reflecting iron sufficiency (IS>NAID>IDA), and although treatment groups' cognitive scores were greater following iron supplementation, the initial differences persisted between iron status groups (Devaki et al., 2009). This may echo how neurocognitive systems are subject to a greater deficit as haematological indices of iron status deplete, which reflects a cognitive capacity that takes longer to correct with iron supplementation. Similarly, improvements to iron status and perceptual, attention and mnemonic abilities are shown following an iron-bio fortified millet intervention at much lower doses than oral iron supplementation forms (Scott et al., 2018).

In contrast to RCTs conducted on cognition in infants, treatment of IDA in older children demonstrates a benefit to cognitive function. It is postulated that older children may respond differently to iron interventions due to the most critical neurodevelopmental stages having occurred in infancy. The advantageous effects of iron supplementation for correcting ID and

IDA in adolescents of both genders could be considered educationally advantageous. The consistent observational findings of ID diminishing scholastic performance and academic achievement combined with the consistent improvements to IQ, attention and memory following iron treatment highlight the importance of maintaining an optimal iron status for reaching cognitive heights during a period of intense scholastic demand.

1.5.4.3 The role of iron in women of reproductive age

Cognitive function is known to naturally decline with age across genders in healthy educated adults starting from their 20s and 30s (Salthouse, 2009). This decline is characterised by near linear declines in speed and accelerating declines in memory and reasoning, despite increases in vocabulary knowledge (Salthouse, 2019). Prevalence of anaemia in non-pregnant women of reproductive age ranges from 9 % to 59 % with prevalence decreasing with economic and social advancements by country (Global Health Observatory, 2020). As >20 % of women experience ID during their reproductive years (Percy et al., 2017), the known cognitive impairments associated with its deficiency in infancy, childhood and adolescence have stimulated an accumulation of evidence regarding altered cognition and depleted iron status in this demographic also.

A significant reduction in haemoglobin following 50 % energy restriction in dieting women of varied iron status who were obese is associated with poorer performance on a task of sustained attention (Kretsch, Fong, Green, & Johnson, 1998). Comparisons between IS and IDA males and females identified significant impairments of memory and IQ with declining iron status (Khedr et al., 2008). Positive associations are also shown in the absence of anaemia between body iron (the log ratio of sTfR to serum ferritin), serum ferritin and executive planning function amongst healthy female university students (Blanton, 2013; Blanton, Green, & Kretsch, 2013). Additionally, greater body iron and serum ferritin was associated with faster response times and improved memory strategy scores on a spatial working memory task (Blanton, 2013). Although lower body iron and ferritin was associated with better immediate free word recall, which is in agreement with an earlier study comparing low and normal ferritin groups (Fordy & Benton, 1994). These findings have been extended to a NAID female population using a higher-difficulty version of the executive function task (Scott & Murray-Kolb, 2016). Iron status groups did not differ in attentional and executive function performance, however a faster planning time was associated with higher serum ferritin and, when age was added as a covariate, higher total body iron. Furthermore, increased body iron and serum ferritin were associated with a smaller change in planning time with increased task difficulty yet worse working memory performance. However, more recent evidence suggests that only

women with IDA exhibit poorer attentional task performance, whereas NAID is not associated with any cognitive differences compared to those who are IS (Cook et al., 2017). Although, NAID females are also shown to have a lower grade point average (GPA) as a measure of academic performance at university, in comparison to IS females (Scott, De Souza, Koehler, & Murray-Kolb, 2017). Together these findings demonstrate the potential benefit of iron for handling higher cognitive loads, although the role of NAID remains inconclusive.

Recent systematic reviews have indicated benefits of iron supplementation on cognitive function; however, findings remain equivocal. The earliest meta-analytic review, regarding older children and women of reproductive age, highlighted improved measures of attention and concentration regardless of baseline iron status over intervention durations of 8-17 weeks; improvements in intelligence were only demonstrated when women were IDA at baseline over intervention durations of 13-29 weeks (Falkingham et al., 2010). When only investigating women of reproductive age, eight out of ten included studies reported cognitive improvements following iron supplementation over 4-20 weeks, though only four studies found that ID at baseline resulted in poorer cognitive performance (Greig, Patterson, Collins, & Chalmers, 2013). Meta-analysis revealed that only arithmetic scores, as a measure of working memory, significantly improved after iron treatment. A subsequent systematic review corroborates and extends these findings; iron supplementation improves memory, specifically working memory, and intellectual ability regardless of baseline ID or IDA (Lomagno et al., 2014). Iron deficiency and IDA were however both shown to be detrimental to cognitive function. An updated systematic review of iron supplementation for cognitive and behavioural function in women of reproductive age is presented in Chapter 3 of this thesis.

More recently, interest has grown regarding dietary interventions rather than conventional oral iron supplementation methods. When investigating the effect of naturally iron-rich beef lunches (~ 3.5 mg/lunch) in comparison to a control lunch, no significant differences in iron status were identified as both groups' iron status improved. However, greater improvements in body iron were demonstrated in women whose body iron was lowest at baseline. Higher body iron and serum ferritin were associated with better planning speed and spatial working memory; these improvements were more pronounced following a serum ferritin response to intervention in comparison to non-responders (Blanton, 2013). The consumption of iron bio-fortified beans (86.1 ppm) by NAID women corroborates and extends these findings (Murray-Kolb et al., 2017). Significant treatment effects were found for speed of attention and speed, efficiency and specificity of memory, whilst a significant increase in serum ferritin was associated with quicker attention and memory task completion. Such studies provide growing evidence for benefits of iron at doses much lower than traditional supplementation methods.

Overall, observational investigations have identified that the importance of iron status for cognitive function is not limited to the developing brain. The iron intervention studies conducted thus far have largely supported this by demonstrating that the cognitive deficits associated with ID can be overcome in women of reproductive age. However, the number of studies specifically investigating iron supplementation that employ non-pregnant women of reproductive age remain scarce despite their risk for NAID and IDA (Percy et al., 2017; Soppi, 2018). In conjunction with the natural age-related decline of cognitive function experienced during this time of life (Salthouse, 2009), ensuring iron sufficiency may be essential for maintaining optimal brain health in pre-menopausal women.

1.5.4.4 The role of iron post-menopause and for neurodegeneration

Women of reproductive age have lower haematological indices of iron compared to middle-aged and elderly women (Cho et al., 2011). A population-based study corroborates this as 11 % of women aged 20-49 present with ID in comparison to 5 % of women aged 50-69 years (Looker, Dallman, Carroll, Gunter, & Johnson, 1997). Longitudinal evidence has identified women having significantly lower serum ferritin and higher sTfR:serum ferritin concentrations during pre-menopause than post-menopause (Kim, Nan, Kong, & Harlow, 2012), presumably due to the cessation of menstrual bleeding. However, as women continue to age post-menopause, anaemia frequency increases where by the age of 85 years, approximately 20 % are considered anaemic (Friedman et al., 2012). Although anaemia may not be due to ID; it becomes increasingly challenging to determine as we age due to age-related changes in haemoglobin, increases in serum ferritin due to chronic activation of inflammatory pathways and the potential effects of medications prescribed for age-related illnesses (Fairweather-Tait, Wawer, Gillings, Jennings, & Myint, 2014). Considering the growing ageing population, iron status and supplementation studies have grown in importance to ensure cognitive function stability and subsequent quality of life.

Strong associations between anaemia and poorer cognitive and physical functions are shown amongst geriatric populations (Denny, Kuchibhatla, & Cohen, 2006), with global cognitive function and episodic memory being highlighted as susceptible (Qin et al., 2019). When specifically investigating ID, cognitive scores were significantly lower for those with ID and the prevalence of dementia was higher (Yavuz et al., 2012). Non-anaemic ID was associated with significantly lower cognitive scores in comparison to those who were IS indicating that the influence ID has on cognitive function may be independent of anaemia in the elderly also. Iron treatment for ID with and without anaemia in an elderly general population demonstrates

significant improvements in cognitive functions and daily functioning (Selvi Öztorun et al., 2018). Considering the demographic was heavily populated with comorbidities, outcomes such as muscle weakness and motor function that are often affected as a result of such comorbidities were improved following iron treatment. The effects of ID with and without anaemia and the positive effects of iron treatment may therefore persist over the lifespan. However, it is essential that underlying causes are identified and treated prior to iron treatment administration due to the natural accumulation of iron in the brain with ageing.

Brain iron dysregulation is observed in the ageing brain, observed as region-specific iron increases in the cerebral cortex, cerebellum, hippocampus, amygdala and basal ganglia white matter (Ashraf, Clark, & So, 2018). The accumulation of toxic levels of iron within these brain regions may cause cell death via apoptosis, autophagy or ferroptosis (Dixon et al., 2012) or proliferate a pro-inflammatory environment that together stimulate neurodegeneration, neuroinflammation, protein aggregation and neurobehavioural deficits observed in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Ward, Zucca, Duyn, Crichton, & Zecca, 2014; Xu, Jia, Knutson, & Leeuwenburgh, 2012). However, the neurological detriments of high brain iron are not restricted to clinical populations. In healthy older individuals, higher hippocampal iron was associated with worse verbal working memory performance in men but not in women (Bartzokis et al., 2011). As women naturally have lower peripheral iron compared to men (Whitfield, Treloar, Zhu, Powell, & Martin, 2003), this may lead to reduced brain iron at older ages meaning that men may be more susceptible to neurodegeneration at earlier ages (Moisan et al., 2016; Ullah et al., 2019). Regardless of gender, iron accumulation in the putamen predicts greater rates of cognitive decline (Daugherty & Raz, 2016) as does striatal iron accumulation alongside changes in verbal working memory (Daugherty, Haacke, & Raz, 2015). Together, this evidence may suggest that iron supplementation is beneficial when ID with or without anaemia is being treated, however providing iron supplementation to the oldest populations presenting with neurodegenerative issues may exacerbate the effects of cognitive decline. It could be argued that iron supplementation is more beneficial for women pre-menopause and is more essential from infancy into the reproductive years to maintain long-term brain health.

1.5.5 Summary of the behavioural effects of iron

Research investigating the effects of iron on behavioural outcomes in both animals and humans has demonstrated that its deficiency leads to a variety of behavioural and cognitive impairments. Iron deficiency is associated with mood and behavioural disorders including depression, anxiety, autism and ADHD, as well as increased fatigue and poorer sleep quality.

However, iron supplementation trials in humans are less conclusive regarding ameliorating these associated impairments across the lifespan. The promising findings thus far warrant continued investigation as research has highlighted the importance of iron for optimal brain functioning across the lifespan. The critical involvement of iron for multiple aspects of neurodevelopment in infancy and childhood is well described alongside research supporting the importance of iron sufficiency during pregnancy for infant neurodevelopment. Furthermore, ID is shown to be detrimental to cognitive function at any time of life, including older age in the presence of comorbidities. However, iron also acts as a double-edged sword as its increased dysregulation with age can promote neurodegeneration. Iron is also important for cognition during early adulthood, specifically for non-pregnant women of reproductive age. Unfortunately, studies are limited within the research area as this demographic are overlooked in favour of those deemed at a higher risk of deficiency. Yet beyond infancy, neural structures are still amenable to an iron insult, which may be detrimental considering the suggestion that cognitive function naturally declines in the 20s and 30s. Within this sample, RCTs have identified benefits to treating ID-associated cognitive deficits and as women of reproductive age are at a prolonged risk of ID due to years of menstruation and the potential of pregnancy, they are at risk of ID with or without anaemia for a large proportion of their adult life. It is therefore imperative that nutritional iron intervention studies investigating a range of outcomes relating to brain health are furthered focussing on women of reproductive age.

1.6 Previous limitations of randomised clinical trials

There are many aspects to consider regarding iron supplements prior to commencing investigations of their use in human trials, most of these revolve around dosage and duration, which have consequences for absorption, tolerability and efficacy. Oral iron supplements are the gold-standard treatment for ID and are available as either solid or liquid preparations, with several variations. Ferrous salts, including ferrous fumarate, ferrous gluconate and specifically ferrous sulphate are the most common form of iron supplements currently in use in the United Kingdom and are considered the first-line treatment for IDA (Goddard, James, McIntyre, & Scott, 2011; Zariwala, Somavarapu, Farnaud, & Renshaw, 2013). However, efficient iron supplement absorption is a complicated process as it is amenable to considerable variability and is often associated with gastrointestinal side effects reducing tolerability and compliance (Tolkien, Stecher, Mander, Pereira, & Powell, 2015). Factors such as sample population, baseline iron status, dietary and lifestyle factors, dose, and study duration, all have the potential to affect the efficacy of iron supplementation, which makes it difficult to compare the findings from different studies due to heterogeneity surrounding the measurement of these factors. These factors are discussed below.

1.6.1 Sample population

A large majority of previous iron supplementation research focusses heavily on either infants, children or pregnant women putting very little focus on healthy, non-pregnant women of reproductive age. This demographic is of particular interest as they are at a prolonged risk for ID due to physiological losses of iron posing demands on its effective absorption, which may be exacerbated by the quantity and quality of dietary iron intake. In Europe, 61-97 % of women of reproductive age have a dietary iron intake below the recommended daily allowance of 14.8-16.0 mg/day (Milman, 2019). Consequently, the demand for iron may be greater than the quantity of iron acquired from the diet, increasing the likelihood of ID despite otherwise being healthy. Not only does this reiterate that ID is the most prevalent nutritional deficiency but also supports the notion of the general population of upper-middle and high-income countries being deficient in one or more vitamins and minerals (Kennedy, 2016; Kennedy & Haskell, 2011). Previous multivitamin intervention studies utilising a healthy, young female population have identified a significant attenuation of the negative effects on physical tiredness following a difficult multi-tasking cognitive battery alongside improvements in accuracy and speed of cognitive functioning (Haskell et al., 2010). Acute and chronic supplementation is also shown to modulate cerebral blood flow and metabolic parameters of energy expenditure during cognitive task performance (Kennedy et al., 2016). It is therefore suggested that both metabolism and brain function are receptive to micronutrient supplementation even when healthy adult females are assumed to have a sufficient nutritional status. As iron is essential for fundamental biological processes that affect psychological and physiological functioning, it is suggested that otherwise healthy females classed as iron deficient or those with an inadequate dietary iron intake regardless of iron status would both benefit from iron supplementation.

Similarly, this population is subject to natural age-related cognitive decline beginning in their 20s or 30s (Salthouse, 2009; Salthouse, 2019), which may be accompanied by age-related alterations in cerebral blood flow inciting a negative association between cerebral blood flow and task performance (Bertsch et al., 2009; Catchlove et al., 2018). Iron deficiency anaemia is associated with an increased cardiac workload, which is shown to invoke compensatory increases in cerebral blood flow to enhance cerebral oxygen delivery when haemoglobin is diminished in infancy (Aliefendioglu et al., 2007; Hare, 2004). However, serum ferritin is shown to moderate relationships between task difficulty and brain activity as measured by electroencephalography (EEG) in women of reproductive age with NAID (Wenger, DellaValle,

Murray-Kolb, & Haas, 2019). This suggests that brain function can still be affected prior to the development of IDA, which may exacerbate the natural age-related cognitive declines.

Considering this, it is not solely IDA that is considered a clinical issue; statistics from the United States indicate that 3-5 % of non-pregnant women aged 16-49 have IDA whilst 11-20 % have NAID (Soppi, 2018; Umbreit, 2005). Similarly, although epidemiological data is scarce for this demographic in the United Kingdom, the prevalence of IDA is estimated to be 5 % whilst the prevalence of NAID may be up to 15 % (SACN, 2010; PHE, 2014). Non-anaemic ID arises in females when haemoglobin concentrations are normal (> 120 g/L) but ferritin stores are depleted. Symptoms remain the same as for IDA, however it poses a clinical challenge in comparison as there are no well-defined diagnostic criteria for a serum ferritin cut-off due to its APP properties. A serum ferritin cut-off of < 15 µg/L is a proposed indicator of ID in healthy female populations (Garcia-Casal, Pasricha, Martinez, Lopez-Perez, & Peña-Rosas, 2018); however, it is arguably too low as serum ferritin 15 - 30 µg/L (Pasricha et al., 2010) and 25 - 40 µg/L (Hallberg et al., 1993) are shown to be highly suggestive of ID. Serum ferritin < 30 µg/L is associated with increased diagnostic accuracy in comparison to a cut off of < 12 µg/L (Mast, Blinder, Gronowski, Chumley, & Scott, 1998), however it is also suggested to increase the occurrence of false-positive ID diagnoses (Daru et al., 2017). Together with the indication that mobilisation of iron stores and their subsequent depletion is reflected by serum ferritin 13-20 µg/L (Langley-Evans, 2013), a cut-off of ≤ 20 µg/L should be considered when investigating NAID. There is promising evidence to suggest a causal role for iron in cognitive function concerning attention, memory and learning domains (Leonard, Chalmers, Collins, & Patterson, 2014; Murray-Kolb & Beard, 2007) and objective measures of athletic performance (Rubeor et al., 2018) when using a serum ferritin cut-off of ≤ 20 µg/L for NAID women of reproductive age. As a reduction in physical capacity often presents simultaneously alongside symptoms of central fatigue characterised by subjective feelings of reduced energy and motivation, which may instigate augmented anxious and depressive emotions that deplete quality of life and cognitive capacity (Dziembowska et al., 2019), further investigation of behavioural function at this serum ferritin cut-off is warranted.

Overall, although reviews have highlighted the beneficial effect of iron supplementation for women of reproductive age, NAID is deserving of further investigation among this population using a serum ferritin cut-off of 20 µg/L. It is postulated that NAID women should still benefit from iron supplementation, as should those who do not consume sufficient dietary iron.

1.6.2 Dosage and duration of iron supplementation

In NAID women, fractional absorption of iron is shown to be highest at lower doses of 40-80 mg in comparison to higher doses of 160-240 mg, with consecutive-day and twice-daily dosing reducing iron bioavailability at high doses (Moretti et al., 2015). Although, alternate-day dosing of iron ranging from 100-200 mg is shown to optimise iron absorption in NAID and IDA women (Stoffel et al., 2017; Stoffel, Zeder, Brittenham, Moretti, & Zimmermann, 2019). A comparative study of iron doses has identified a 60 mg iron dose to be as effective as an 80 mg dose for improving iron status in NAID women with no significant difference in adverse events or compliance (Leonard et al., 2014). However, 60 mg is substantially greater than the recommended daily allowance (RDA) of 14.8-16 mg for non-pregnant, menstruating women (Milman, 2019), and greater than the tolerable upper level of iron intake of 40-45 mg based upon the prevention of gastrointestinal discomfort (Pra, Franke, Henriques, & Fenech, 2012). A systematic review highlighted a significant association between increased gastrointestinal side effects and iron doses exceeding 30 mg elemental iron (Low, Speedy, Styles, De-Regil, & Pasricha, 2016). Resolution of NAID has however been achieved after an 8-week intervention of 26 mg daily iron supplementation without the occurrence of adverse effects (D'Adamo, Novick, Feinberg, Dawson, & Miller, 2018). Although serum ferritin improved from baseline, this was only to a mean of 21.1 µg/L, which is only marginally above the 20 µg/L cut off for NAID classification. However, the 8-week intervention period was below the recommended duration of three to six months for iron supplementation to effectively restore iron levels (Baird-Gunning & Bromley, 2016), which may explain why the improvement in iron status was not pronounced. A lower dose supplement over a longer duration may therefore be more beneficial for achieving an optimal iron status and reducing adverse effects to ensure compliance.

1.6.3 Increasing the absorption and tolerability of iron supplements

Although oral iron supplementation is the gold-standard treatment for ID, specifically ferrous sulphate forms, its daily administration in a non-clinical population is associated with an increased risk of gastrointestinal side effects regardless of dose when compared to placebo or intravenous iron delivery, which lead to reductions in treatment compliance (Tolkien et al., 2015). Intravenous iron however is only used under certain circumstances such as known iron malabsorption, cancers, chronic kidney disease, inflammatory bowel disease, pregnancy, an urgent need to raise haemoglobin concentrations and poor compliance due to intolerable side effects of oral iron (Gozzard, 2011; Singh, Fong, & Kuperan, 1998). Using intravenous iron in RCTs for non-clinical samples would lack ecological validity, therefore alternate oral forms of iron are deserving of investigation.

To improve iron supplementation tolerability, alternate preparations including polynuclear formulations based on the ferric iron form, such as iron hydroxide polymaltose complex (IPC) (Yasa, Agaoglu, & Unuvar, 2011), have been investigated. A meta-analysis highlighted oral IPC to be as effective as ferrous sulphate formulations for improving haemoglobin concentrations, whilst reducing the prevalence of adverse side effects (Toblli & Brignoli, 2007). A trial in adolescents of both sexes with varied iron status used oral IPC containing 100 mg elemental iron given six days a week for eight months; no adverse side effects were reported whilst concomitantly improving haematological parameters and cognitive and scholastic performance (Devaki et al., 2009). Iron sufficient, NAID and IDA groups all elicited significant increases in serum ferritin; however, IS adolescents who had higher initial haemoglobin concentrations compared to NAID and IDA adolescents had the highest gain of serum ferritin. These findings are corroborated by recent data that discovered no correlation between initial haemoglobin concentrations and increases in serum ferritin in IDA children administered oral IPC (Name, Vasconcelos, & Valzachi Rocha Maluf, 2018). It is suggested that oral IPC may therefore encourage the development of iron overload.

Iron bis-glycinate chelate compounds are a suggested resolution to counteract the concerns associated with bivalent ferrous supplements and ferric IPC preparations. The bis-glycinate chelate consists of two heterocyclic rings (Figure 1.6) orientated at uniform tetrahedral angles in the least sterically hindered conformation possible (Ashmead, 2001). This conformation protects iron from interacting with dietary inhibitors including calcium, phytates and polyphenols to form insoluble compounds increasing the bioavailability of iron for absorption. The five-member rings infer a stronger stability constant than that of inhibitory ligands, which have the potential to displace the ferrous cation from the glycine ligand causing its disassociation in the gut (Ashmead, 2001). Additionally, the chelate has a low molecular weight of 204 daltons (Da); a value much lower than the upper limit of 1,000-1,500 Da for effective chelate absorption in humans (Ashmead, 2001; Kratzer, 2018). Although greater than ferrous sulphate (152 Da) (National Center for Biotechnology Information, 2019), the chelate is more likely to be successfully absorbed as ferrous sulphate is more susceptible to the detrimental impact of inhibitory ligands. Subsequently, iron bis-glycinate chelate is favoured over bivalent ferrous formulations for iron fortification food and drink strategies to combat ID. Studies have identified greater absorption of fortified iron from bis-glycinate chelate than ferrous sulphate in typically iron inhibiting mediums; fortified whole cow's milk (Olivares et al., 1997; Stekel et al., 1986; Torres, Lobo, Sato, & Queiroz Sde, 1996), polyphenol and phytate-rich breakfasts (Layrisse et al., 2000), phytate-rich breads (Giorgini et al., 2001) and high-phytate whole-maize porridge (Bovell-Benjamin, Viteri, & Allen, 2000).

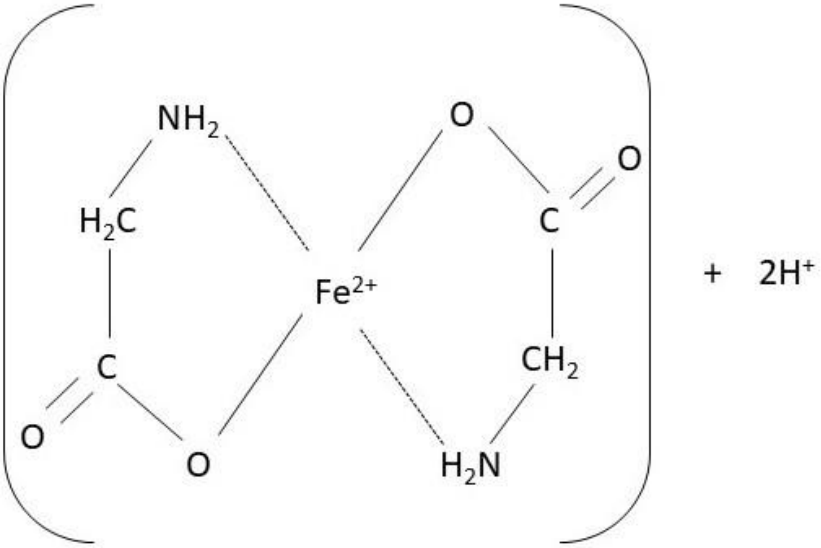


Figure 1.6 Structural formation of iron bis-glycinate chelate ($C_4H_8FeN_2O_4$). The chelate consists of two, five-member, heterocyclic rings comprising two bonds between the ferrous cation and the glycine molecules. The carboxyl group and the α-amino group of the glycine molecule bind the ferrous cation by an ionic bond and a coordinate covalent bond respectively (figure adapted from Ashmead, 2001).

The structural formation of iron bis-glycinate is suggested to promote gastrointestinal tolerability as the amino acid ligand ring formation prevents the ferrous cation from interacting with the mucosal lining (Ashmead, 2001). The lower stability constant of ferrous sulphate is suggested to cause its dissociation in the gut to promote elemental iron release in the stomach, which initiates gastrointestinal irritation and subsequent side effects (Ashmead, 2001). Human studies provide support for this concept; non-pregnant women of reproductive age with IS iron status demonstrated a significantly higher preference for iron bis-glycinate chelate over ferrous sulphate following trends for lower incidence of gastrointestinal adverse effects (Coplin, Schuette, Leichtmann, & Lashner, 1991) and significantly fewer reports of adverse effects (Fouad et al., 2013). This evidence suggesting superior bioavailability and tolerability of iron bis-glycinate has prompted its use as an alternative oral supplement for the treatment of IDA. Amongst pregnant women, lower doses of iron bis-glycinate have initiated significantly less pronounced pregnancy-associated decreases in iron status parameters (Szarfarc, de Cassana, Fujimori, Guerra-Shinohara, & de Oliveira, 2001), significantly lower gastrointestinal complaints (Milman, Jonsson, Dyre, Pedersen, & Larsen, 2014) and a significantly faster rise in haemoglobin (Abdel Moety et al., 2017) in comparison to ferrous salts. Iron bis-glycinate is also shown to improve haematological measures above the IDA cut-off more efficiently than an equal dose of ferrous sulphate in the presence of significantly fewer reported side effects (Abbas, Abdelbadee, Alanwar, & Mostafa, 2019). For infants and

adolescents, lower (Bagna et al., 2018; Pineda, Ashmead, Perez, & Lemus, 1994) and equal doses (Pineda & Ashmead, 2001) of iron bis-glycinate chelate are shown to improve haemoglobin with similar efficacy but serum ferritin with greater efficacy than ferrous sulphate in the presence of fewer adverse effects. Iron bis-glycinate also demonstrates superior efficacy than oral IPC for treatment of IDA in children by increasing iron stores through absorption that is regulated by the demand for iron (Name et al., 2018). This corroborates previous findings of an inverse relationship between iron stores and the absorption of iron bis-glycinate chelate (Bovell-Benjamin et al., 2000; Olivares et al., 1997). From these findings, there is support for iron bis-glycinate chelate supplementation to be of most benefit to a NAID population.

1.6.3.1 Vitamin C co-supplementation

Vitamin C, otherwise known as ascorbic acid, cannot be synthesised by the human body so must also be acquired from the diet (Hansen, Tveden-Nyborg, & Lykkesfeldt, 2014); however, the brain has the ability to recycle it to prevent the neurological symptoms associated with its deficiency, including severe fatigue, confusion, and depression (Caballero, Trugo, & Finglas, 2003). In addition to its well-known anti-oxidant properties, vitamin C is vital for neuromodulation acting as a co-factor for acetylcholine, noradrenaline, dopamine and serotonin synthesis (Figueroa-Méndez & Rivas-Arancibia, 2015; Gupta, Tiwari, & Haria, 2014); modulation of GABA-ergic systems and inhibition of N-methyl-D-aspartate receptors and L-arginine-nitric-oxide cyclic guanosine 3,5-monophosphate pathway (Covarrubias-Pinto, Acuna, Beltran, Torres-Diaz, & Castro, 2015); and inducing haem-oxygenase 1 expression (Gariballa, 2014). Dysfunction of these metabolic processes is linked to depression and anxiety, instilling the hypothesis of ascorbic acid having anti-depressant-like properties. High ascorbic acid status has been associated with improved mood (Prohan, Amani, Nematpour, Jomehzadeh, & Haghighizadeh, 2014; Pullar, Carr, Bozonet, & Vissers, 2018), further supported by intervention trials with oral vitamin C showing significantly reduced depressive, anxiety and fatigue symptoms following 500 mg/day supplemental (de Oliveira, de Souza, Motta, & Da-Silva, 2015) and 212 mg/day dietary vitamin C (Carr, Bozonet, Pullar, & Vissers, 2013) over two and six weeks, respectively. Similarly, significant associations between plasma ascorbic acid and performance on tasks of attention, memory (working, recall and recognition), focus and decision speed have been identified (Travica et al., 2019), mechanisms for which include the known roles of acetylcholine and noradrenaline for attention, focus and memory performance (Klinkenberg, Sambeth, & Blokland, 2011); the impact of hippocampal and prefrontal cortex serotonergic neurons upon memory, decision making, and attention (Švob Štrac, Pivac, & Mück-Šeler, 2016); and the increased synthesis of energy through L-carnitine

production allowing the optimal metabolic energy supply for peak cognitive performance (Owen & Sunram-Lea, 2011).

Vitamin C is also capable of reducing ferric iron from the diet to ferrous iron but can also chelate ferrous iron to preserve its solubility and increase its uptake across the duodenum with a change in pH (Conrad & Schade, 1968). The addition of a vitamin C-rich fruit to a ferrous sulphate-fortified breakfast cereal significantly improved serum ferritin concentrations in women with serum ferritin ≤ 25 µg/L and haemoglobin ≥ 115 g/L (Beck, Conlon, Kruger, Coad, & Stonehouse, 2011). Consequently, combination therapy of iron supplements and vitamin C is often recommended for IDA treatment. However, despite the positive associations between cognition, mood, fatigue and vitamin C, iron and vitamin C co-supplementation has not been previously investigated with regards to the effects on cognitive and behavioural functions. Additionally, to our knowledge, co-supplementation of iron bis-glycinate and vitamin C has not previously been investigated.

The efficacy of iron bis-glycinate chelate (15 µg/L) in an iron inhibiting medium like milk for improving iron status is deemed comparable to the co-fortification of ferrous sulphate and ascorbic acid (Olivares et al., 1997; Stekel et al., 1986). The structural configuration of the chelate protects against inhibitory compounds, whilst the addition of ascorbic acid (100 mg/L) is shown to further stimulate iron bis-glycinate absorption (15 µg/L) in an inhibitor-rich environment (Olivares et al., 1997). Although ferrous sulphate formulations are more amenable to the benefits of ascorbic acid (Olivares et al., 1997), such evidence indicates that the addition of ascorbic acid improves the already superior bioavailability of iron bis-glycinate chelate. In view of this, co-supplementing iron bis-glycinate chelate with ascorbic acid would not only benefit its absorption but would also reduce the impact of inhibitory compounds found in a normal dietary intake.

1.6.4 Dietary and lifestyle factors

As iron cannot be synthesised by the human body, all iron sources are acquired externally through the diet. Iron deficiency and IDA rates are highest in low-income developing countries primarily due to the combination of exposure to chronic infection and limited access to an iron-rich diet (Prentice et al., 2017). Within upper-middle and high-income countries, ID remains the most prevalent nutritional deficiency by consequence of common lifestyle factors that increase deficiency occurrence, including dietary iron consumption and bioavailability, physical activity and menstrual blood loss.

Although possible to achieve optimal iron intake without consuming meat, review findings provide evidence for a positive association between animal flesh intake/day and iron status (Jackson, Williams, McEvoy, MacDonald-Wicks, & Patterson, 2016). Meta-analysis corroborated these findings by concluding that vegetarians have significantly lower serum ferritin concentrations compared to non-vegetarians subsequently increasing the risk for ID (Haider, Schwingshackl, Hoffmann, & Ekmekcioglu, 2018). This may be due to an increased consumption of phytate-rich grains and cereals, polyphenol-rich food and drink, and calcium-rich dairy products that have inhibitory effects on non-haem bioavailability (Beck et al., 2014). Similarly, alternate nutritional deficiencies may contribute to cognitive limitations and affect the efficacy of iron supplementation. Iron and zinc share some absorptive and transport mechanisms so may compete for greater absorption, initiating redundancies in both minerals (Sandstrom, 2001). Iron supplementation can significantly reduce zinc absorption due to this competition (Kordas & Stoltzfus, 2004; Sandstrom, Davidsson, Cederblad, & Lonnerdal, 1985; Solomons & Jacob, 1981) and exacerbate the potential cognitive deficits associated with ID (de Moura et al., 2013; Stoecker et al., 2009). Therefore, it may be imperative to assess dietary iron intake and to monitor zinc levels across intervention periods. A greater risk for depressive and anxiety disorders is attributed to a lower consumption of iron-rich red meat independent of other dietary influences in a sample of adult women (Jacka et al., 2012) whilst vegetarian diets may influence mental well-being due to the loss of nutrients from excluded foods causing an elevation in depressive symptoms compared to omnivores (Baines, Powers, & Brown, 2007; Forestell & Nezlek, 2018; Matta et al., 2018). As iron has a key role in monoaminergic neurotransmitter modulation, this may explain the prevalence of mood dysfunction associated with lower dietary iron intake. Additionally, as absorption of dietary iron can be impacted by multiple variables, dietary iron may have a direct effect upon psychological parameters that is independent of iron status; however, this potentially influential effect has not previously been considered.

Iron deficiency may also arise following acute exercise-induced inflammation upregulating hepcidin expression, which diminishes effective iron absorption (McClung et al., 2014), as discussed in section 1.5.2. Despite an adequate daily dietary intake of iron, trained female endurance athletes exhibit significant hepcidin elevations with increases in training load that increase the risk for ID (Ishibashi, Maeda, Sumi, & Goto, 2017). A systematic review extended these findings to single acute bouts of endurance exercise between 30-120 minutes at moderate or high intensity facilitating the upregulation of hepcidin expression between 0 hours and 6 hours post-exercise, peaking at 3 hours which may coincide with high-iron meals being consumed and not effectively absorbed (Domínguez et al., 2018). For untrained women who partake in regular aerobic exercise, the efficacy of iron supplementation is diminished in

comparison to those who do not regularly exercise (Pompano & Haas, 2017). The aforementioned findings regarding athletes may therefore apply to physically active but untrained women in that iron absorption is reduced due to exercise-induced upregulation of hepcidin and an increased demand of iron for use in oxidative pathways (Buratti, Gammella, Rybinska, Cairo, & Recalcati, 2015). Alternatively, poorer physical performance is also associated with IDA and NAID amongst females (Brownlie IV, Utermohlen, Hinton, Giordano, & Haas, 2002; Brutsaert et al., 2003; Haas, Seymour, Hernandez, Dehaene, & Villalpando, 2002; Haas & Brownlie IV, 2001; Hinton, Giordano, Brownlie, & Haas, 2000; Zhu & Haas, 1998) alongside lower physical activity; NAID females spend more time in sedentary behaviours than those who are IS, which potentially contributes to body mass gain (Crouter, DellaValle, & Haas, 2012). Increased adipose tissue induces augmented pro-inflammatory cytokine and hepcidin production, which attenuates dietary iron absorption due to increased ferroportin degradation (Aigner, Feldman, & Datz, 2014; Tussing-Humphreys, Pusatcioglu, Nemeth, & Braunschweig, 2012). Obesity-associated systemic inflammation is more pronounced in females than males and this has been associated with poor cognitive performance (Cook et al., 2017; Gimeno, Marmot, & Singh-Manoux, 2008; Sweat et al., 2008), depression (Amiri, Behnezhad, & Nadinlui, 2018; de Wit et al., 2010; Evangelou et al., 2019), daytime sleepiness, sleep quality, and general fatigue thus influencing ratings of quality of life (Jarosz et al., 2014; Vgontzas et al., 1997). In the United Kingdom, 40 % of adult females are insufficiently active and exhibit lower levels of physical activity in comparison to males (Guthold, Stevens, Riley, & Bull, 2018). Failure to meet physical activity recommendations is associated with greater levels of fatigue in women; effects that are exacerbated by prolonged sedentary behaviours (Ellingson, Kuffel, Vack, & Cook, 2014). A systematic review concluded that physical activity has a positive effect on cognitive functioning (Cox et al., 2016) and mood (Rebar et al., 2015) in young to middle-aged adults due to modulation of monoaminergic neurotransmitters (Strasser & Fuchs, 2015), the production of which may be downregulated by ID (Beard & Connor, 2003). Yet, despite the influence of physical activity and obesity upon iron status, cognitive function, mood and fatigue, only one cross-sectional study assessing the impact of iron status on cognition has accounted for the confounding variability (Cook et al., 2017), whilst no studies investigating mood and fatigue have done so.

Blood loss through regular blood donation is a known risk factor for ID in both males and premenopausal women (Beck et al., 2014; Reddy, Shastry, Raturi, & Baliga B, 2020); however, women of reproductive age are at an additional risk for ID due to increased iron losses because of heavy menstrual bleeding (Johnson, Lang, Sturm, & O'Brien, 2016). Quantitative measures of menstrual blood loss have been shown to significantly predict iron status (Harvey et al., 2005), whilst subjective approaches have indicated significantly lower

serum ferritin concentrations for those with greater menstrual blood loss (Heath, Skeaff, Williams, & Gibson, 2001; Toxqui, Pérez-Granados, Blanco-Rojo, Wright, & Vaquero, 2014). Haemoglobin and serum ferritin concentrations are also lowest during menses and highest during the luteal phase of the menstrual cycle (Kim, Yetley, & Calvo, 1993). This suggests that physiological biomarkers of iron undergo cyclical variations, thus it is recommended to assess such biomarkers during the late luteal phase of the menstrual cycle for an accurate representation of iron status (Laine et al., 2016) as well as taking menstrual blood loss into consideration. Irrespective of ID, increased menstrual blood losses affect physical health, fatigue, well-being and productivity (Copher et al., 2012; Karlsson, Marions, & Edlund, 2014; Kim et al., 1993; Kocaoz, Cirpan, & Degirmencioglu, 2019; Wang et al., 2013), which instil an increased degree of limitations in physical, social and leisure activities affecting quality of life (Lukes, Baker, Eder, & Adomako, 2012). Despite such evidence, objective and subjective assessments of menstrual blood loss have not been considered when investigating the associations between iron status parameters and psychological function. Similarly, women's serum ferritin concentrations are at their lowest between 25 and 35 years of age and are generally lower until post-menopause where they abruptly increase (Chełchowska, Laskowska-Klita, & Leibschang, 2007). When considered alongside the fact that cognitive function is known to naturally decline with age in healthy educated adults starting from their 20s and 30s (Salthouse, 2009; Salthouse, 2019), it may be imperative to also consider age when investigating the associations between iron status parameters and psychological function.

1.7 Rationale and aims of the thesis

The foregoing literature review establishes the involvement of iron in numerous fundamental cellular processes, specifically regarding neurobiological functioning. As iron is only attainable through dietary sources, its involvement in these processes is considered essential for maintaining cellular homeostasis with both ID and overload having detrimental effects upon physiological and behavioural outcomes. As ID is the most prevalent nutritional deficiency worldwide, previous RCTs have focussed on its impact but are limited by methodological issues including classification of iron status, supplement dosage and duration as well as disregarding important factors such as supplement tolerability and bioavailability and lifestyle factors that can affect iron status regardless of iron supplementation.

The evidence evaluated in the literature review highlights the need for further research investigating the effects of iron status and the effects of supplementation on women of

reproductive age as they are a population that has been largely overlooked in favour of those considered to be at a higher risk for ID. The combination of prolonged iron losses from menstruation, the potential for pregnancy associated iron losses and the likelihood of an inadequate dietary iron intake bolster the need for further investigations. As NAID is more prevalent among this population than IDA, NAID warrants further investigation. Serum ferritin-dependent associations with fatigue are becoming increasingly apparent; however, findings remain equivocal regarding mood, wellbeing and cognition, whilst there is limited data available regarding sleep quality. Previous RCTs have shown promising effects of iron supplementation; however, should positive physiological or behavioural changes be observed in NAID women of reproductive age with a lower and more tolerable dose of iron then these results may offer insights into recognising and treating NAID before progression to IDA.

Accordingly, the primary purpose of this thesis is to investigate iron status and the effects of iron bis-glycinate chelate and iron bis-glycinate chelate/vitamin C co-supplementation on cognitive function, subjective fatigue, mood and wellbeing in NAID menstruating women of reproductive age. Overall, this thesis intends to address the following aims:

1. Investigate association of iron status, haemoglobin concentrations and serum ferritin concentrations with cognition, subjective mood, fatigue and wellbeing in menstruating women of reproductive age aged 18-49 years (Chapter 2).

2. Systematically review current understanding of findings from RCTs investigating the effects of iron supplementation on cognition, subjective mood, fatigue and/or wellbeing in menstruating women of reproductive age aged 18-49 years (Chapter 3).

3. Investigate the effect of iron bis-glycinate chelate and iron/vitamin C co-supplementation on cognition, subjective mood, fatigue and wellbeing in menstruating women of reproductive age aged 18-49 years (Chapter 4).

4. Investigate the effect of iron bis-glycinate chelate and iron/vitamin C co-supplementation on cerebral blood flow and energy expenditure at rest and during cognitive demand in menstruating women of reproductive age aged 18-49 years (Chapter 5).

The experimental investigations within this thesis will be the first employing both iron bis-glycinate chelate alone and co-supplemented with vitamin C upon cognition, subjective mood,

fatigue and well-being. Additionally, assessing the effects of iron supplementation on cerebral blood flow is novel to the research area.

Chapter 2 **THE ASSOCIATION OF IRON STATUS, HAEMOGLOBIN AND SERUM FERRITIN TO COGNITIVE FUNCTION, MOOD, FATIGUE AND WELL-BEING IN WOMEN OF REPRODUCTIVE AGE**

2.1 Introduction

Non-anaemic iron deficiency (NAID) is a clinical challenge worldwide for women of reproductive age. The psychological differences between women who are iron deficient anaemic (IDA) or iron sufficient (IS) are well established; IDA is associated with an increased likelihood of cognitive, emotional and behavioural dysfunction (Jáuregui-Lobera, 2014), however the differences between NAID and IS remain ambiguous. The functional role of iron for cognitive performance is often attributed to its essential role in meeting the metabolic demands of oligodendrocytes for axon myelination. Iron deficiency (ID) is postulated to induce myelin deficits across the lifespan (Oberman & Pascual-Leone, 2013) reducing neural information processing speeds, which are essential for maintaining attention, learning, memory and planning cognitive functions (Nickel & Gu, 2018). However, studies remain limited to the populations considered at a higher risk of ID such as infants, children and pregnant women whilst the prolonged risk to non-pregnant women of reproductive age due to years of menstruation and the possibility of pregnancy is often overlooked.

Cross-sectional studies comparing the cognitive function of NAID and IS women of reproductive age have shown no significant differences across iron status groups for attention and executive function (Scott & Murray-Kolb, 2016). Similarly, previous iron intervention studies focussed upon group comparisons between NAID and IS women of reproductive age have found no significant differences in baseline cognitive performance (Leonard et al., 2014; Murray-Kolb & Beard, 2007). Those who are NAID and IS have demonstrated superior cognitive performance in comparison to those who are IDA (Murray-Kolb & Beard, 2007), in agreement with more recent evidence that identified no cognitive differences between NAID and IS women yet greater attentional task performance than IDA women (Cook et al., 2017). Despite this, NAID women are shown to have a lower grade point average (GPA) as a measure of academic performance in comparison to those who are IS (Scott et al., 2017). Overall, the findings are equivocal as to whether NAID infers cognitive dysfunction in comparison to IS women of reproductive age.

In contrast, cross-sectional studies of women of reproductive age without anaemia that have instead investigated continuous markers of iron status have identified significant associations with cognitive function. Increased body iron and serum ferritin were associated with faster

executive function planning times and smaller changes in planning time with increased task difficulty (Blanton, 2013; Blanton et al., 2013; Scott & Murray-Kolb, 2016). However, findings are mixed regarding memory; both positive (Blanton et al., 2013) and negative associations (Scott & Murray-Kolb, 2016) of body iron and serum ferritin with working memory performance have been identified in addition to negative associations with immediate word recall (Blanton, 2013). When compared to iron status group comparisons, investigating individual continuous markers of iron status highlights potential benefits of iron for handling higher cognitive loads, though further investigation is required to determine the direction of such associations across all cognitive domains.

As stated in section 1.5.1, in addition to ID-associated cognitive deficits, women naturally suffer from detriments to the monoaminergic system in comparison to men (Jovanovic et al., 2008; Staley et al., 2006) and are at a greater risk for ID. Iron may contribute to the aetiology of depression as indicated by the association of IDA with increased reports of depressive symptoms in women (Pamuk et al., 2015; Vahdat Shariatpanaahi et al., 2007). However, most cross-sectional studies within this area have focussed upon women with postpartum depression (Beard et al., 2005; Sheikh et al., 2017; Wassef et al., 2019) regardless of the prevalence of one in 20 non-pregnant women of reproductive age experiencing major depression (Guo, Robakis, Miller, & Butwick, 2018). Those that have investigated non-pregnant women have shown equivocal results. The frequency of NAID is 15 % greater in women with depression and serum ferritin has been shown to be 11 µg/L lower in those with depressive symptoms compared to healthy women (Vahdat Shariatpanaahi et al., 2007). Yet recent evidence suggests no independent relationships between haemoglobin, serum ferritin and depression and that iron deficient serum ferritin (< 20 µg/L) is only associated with depressive symptoms when concomitant fatigue is reported (Price, Abernathy, Dobbs, & Gallaher, 2017). Such ambiguity highlights the need to further investigate a sample of non-pregnant women of reproductive age.

The role of haemoglobin for oxygen transport is often proposed as the main cause of fatigue onset in ID as reduced haemoglobin diminishes the amount of oxygen delivered to the tissues. However, as haemoglobin is unaffected in NAID, alternate mechanisms must be involved. As explained in section 1.5.2, ID is characterised by augmented serotonin and decreased dopamine, thus fulfilling the monoamine central fatigue hypothesis. Meta-analysis of cross-sectional studies evinced significant associations between NAID and subjective fatigue in women of reproductive age but only in populations screened for randomised controlled trials recruiting participants with a primary concern of fatigue or with diagnosed disorders and not within the general population (Yokoi & Konomi, 2017) despite consistent reports of women

within the general population having higher fatigue scores than men (Cullen et al., 2002; Engberg et al., 2017; Ridsdale et al., 1993). A stronger association is to be expected when assessing a population who already recognise their fatigue and so further investigations are warranted amongst a general population of women of reproductive age.

Although NAID is more common than IDA and is becoming more prevalent amongst women of reproductive age, no well-defined diagnostic criteria exist (Soppi, 2018). Such disparity across studies assessing cognitive and behavioural function may be a consequence of categorising based upon varied diagnostic criteria. Worldwide clinical guidelines suggest anaemia can be diagnosed in women of reproductive age when haemoglobin is <120 g/L and a serum ferritin cut-off of 15 µg/L is indicative of ID (World Health Organization, 2017), whilst clinical laboratories implement a reference range of 10-20 µg/L (Goodnough, Nemeth, & Ganz, 2010). However, serum ferritin 15-30 µg/L is highly suggestive of ID (Pasricha et al., 2010) with diagnostic accuracy of serum ferritin increasing from 25 % to 92 % when changing from a cut-off of 12 µg/L to 30 µg/L respectively (Mast et al., 1998). Categorising participants into groups based upon inconsistent diagnostic criteria may conceal the impact of ID, especially effects that can be attributed to haemoglobin and serum ferritin amongst NAID populations. Equally, categorising continuous iron biomarkers into iron status groups may cause unequal sample sizes, loss of power and increased heterogeneity between the groups. This may explain the mixed findings regarding cognitive function across studies using a NAID population as when continuous iron biomarkers are assessed, potential benefits of iron for handling higher cognitive loads (Blanton, 2013; Blanton et al., 2013; Scott & Murray-Kolb, 2016) are observed.

Additionally, the mixed findings regarding the impact of iron upon psychological parameters may be because of little consideration for certain lifestyle factors that increase ID occurrence including physical activity, body mass index (BMI), dietary iron consumption and menstrual blood loss as discussed in section 1.6.4. The evidence regarding how NAID affects cognitive and behavioural function is largely mixed. As no well-defined diagnostic criteria exist for NAID, grouping participants into iron status groups using various cut-offs for both serum ferritin and haemoglobin increases the heterogeneity within and across study samples. This implies the need to investigate the biomarkers of iron status as continuous variables to determine independent associations, in addition to the categorical iron status groupings. Additionally, physical activity, BMI, dietary iron, menstrual blood loss and age all have the potential to affect iron status biomarkers, as well as potentially having direct effects on cognitive function, mood, fatigue and well-being, yet there is a lack of control for such variables across previous studies. The present study therefore aims to investigate the associations of categorical iron status

groups, and continuous biomarkers of iron status haemoglobin and serum ferritin to psychological function in women of reproductive age, whilst controlling for the potential confounding effects of demographic and lifestyle factors.

2.2 Methods

2.2.1 Design and ethics

This study employed an observational, cross-sectional design. Ethical approval was gained from Northumbria University's Psychology Department and the study was conducted according to the Declaration of Helsinki (1964). The study was registered on www.clinicaltrials.gov under the identifier NCT04257669.

2.2.2 Participants

Four hundred and fourteen females aged 18-49 years were recruited. All participants were recruited via advertisement posters around Northumbria University and local businesses, advertisements on social media and intranet services or by emails sent to university staff and students as well as those on the Brain, Performance and Nutrition Research Centre's (BPNRC) participant database. All participants therefore lived in the surrounding area of Newcastle-upon-Tyne. All participants enrolled were aged between 18-49 years, self-reported that they were in good health and had a BMI between 18.5 and 40 kg/m^2. A wide BMI range was chosen to include participants that may have elevated hepcidin levels as a result of obese adipose tissue that may increase the risk of ID (Aigner et al., 2014; Tussing-Humphreys et al., 2012). Participants were able to participate if they were proficient in English, a non-smoker, free from major illnesses, free from any blood disorders excluding anaemia and were not taking any prescription medications excluding the contraceptive pill. To ensure an accurate measure of iron status parameters, participants were excluded if they regularly used dietary or herbal supplements, had used iron supplements within the past four months or had donated more than 300 ml of blood within the past 3 months. Participants were only eligible to take part if the phlebotomist could obtain venous blood samples on the day of screening. An a priori power analysis was conducted to calculate the sample size based on a small-medium effect size (r = 0.2) observed in a previous observational study (Blanton et al., 2013). This indicated that a total sample of 193 participants was required to detect significant associations at an alpha level of 0.05 to achieve power of .80. In comparison to previous observational studies (Blanton et al., 2013; Cook et al., 2017; Scott et al., 2017; Scott & Murray-Kolb, 2016), the expected sample size was deemed sufficient. Additionally, as the current study was fundamental in recruiting participants into the study described in Chapter 4, participants continued to be recruited to the current study until the required sample size was achieved in the subsequent study.

2.2.3 Demographic/lifestyle measurements

2.2.3.1 Caffeine consumption questionnaire (CCQ) (BPNRC, Northumbria University)

The Caffeine Consumption Questionnaire (CCQ) is a questionnaire developed in-house to calculate participants' estimated typical daily caffeine consumption (mg/day) (APPENDIX I). Participants are required to indicate the caffeinated products (fresh brewed coffee; instant coffee; decaffeinated coffee; black/green/white tea; decaffeinated black/green/white tea; cocoa drink; cola; Lucozade products; red bull; monster/relentless/rockstar; solid chocolate bar; square of solid chocolate), if any, that they would have at particular times of the day (breakfast; between breakfast and lunch; lunch; between lunch and dinner; dinner; after dinner) and the most applicable portion size of the product as indicated by picture graphics. Total caffeine consumption (mg/day) is calculated by totalling the caffeine contained in each of the indicated products.

2.2.3.2 International physical activity questionnaire short form (IPAQ) (Craig et al., 2003)

The International Physical Activity Questionnaire (IPAQ) measures the types of intensity of physical activity that participants do as a part of their daily lives to give a metabolic (MET)-minutes score. Participants are required to estimate how often during the last 7 days they have taken part in vigorous and moderate physical activities and walked for at least 10 minutes at a time. Continuous MET-minute/week scores are calculated by multiplying the average MET value of the activity (8, 4 and 3.3 for vigorous, moderate and walking activities respectively) by the number of activity minutes and days. The IPAQ is a reliable and valid measure of physical activity across multiple countries in comparison to other self-report measures (Craig et al., 2003).

2.2.3.3 Menstrual cycle and blood loss questionnaire

This questionnaire (APPENDIX II) was adapted from a published menstrual blood loss (MBL) questionnaire (Toxqui et al., 2014). Participants were required to detail whether they used hormonal contraceptive methods and whether they had had a period or withdrawal bleed in the last three months. Information was also gathered regarding the date of onset of their last menstrual bleed, usual number of days menstruation, estimated number of heavy blood loss days and the estimated date of onset of their next menstrual bleed. Additionally, participants

were required to specify the number and type of pads and/or tampons used during their heaviest blood loss day of their bleed, both during the day and night. Menstrual blood loss was estimated by multiplying the relative absorbance values of the different pads and tampons by the number of units used by the participant; a method deemed reliable (α = 0.83) for menstrual blood loss estimation (Toxqui et al., 2014). An MBL-score was then calculated taking into consideration the number of heavy days, number of days menstruation and the estimated MBL using the following formula in Figure 2.1:

$$\text{MBL-score} = \left(\frac{\text{Number of heavy days}}{\text{Number of days menstruation}} \right) \times \text{MBL}$$

Figure 2.1 Formula used to create the MBL-score from the menstrual cycle and blood loss questionnaire (Toxqui et al., 2014).

2.2.3.4 EPIC-Norfolk food frequency questionnaire (FFQ)

The EPIC-Norfolk food frequency questionnaire (FFQ) consists of 130 food items, which participants had to select the most appropriate frequency of consumption over the last year ranging from ‘never or less than once/month’ to ‘6+ per day’. An additional six questions were asked regarding milk type and frequency, cereal type, fat for frying, fat for baking, and consumption of visible fat. The FFQ is processed by FETA software (Mulligan et al., 2014) to calculate a daily nutrient and food weight producing an output of 46 nutrients and 14 food groups consumed daily in mg and g, respectively. For the purpose of this thesis, dietary iron was the only nutrient utilised in analysis. Iron-relevant food groups, meat and meat products; fish and fish products; cereal and cereal products; nuts and seeds; and vegetables were used for demographic information.

2.2.4 Computerised cognitive assessments

The Computerised Mental Performance Assessment System (COMPASS, Northumbria University, Newcastle-upon-Tyne, UK) was used to administer all cognitive assessments and visual analogue scales across all studies included in the thesis. The tasks included are well-validated and have previously demonstrated sensitivity to a range of acute and chronic nutritional interventions in healthy adults (Haskell-Ramsay et al., 2018; Kennedy et al., 2017; Stonehouse et al., 2013). Cognitive task responses were registered by Cedrus RB-530 response pads with the exception of the word recall, serial subtractions and peg and ball,

which were instead recorded by pen and paper, keyboard entry and mouse clicks, respectively. The cognitive tasks are described below in order of presentation.

2.2.4.1 Computerised location learning task (cLLT)

Participants were shown a 5 x 5 grid containing pictures of objects and were asked to remember the location of the objects as accurately as possible. The presentation duration was 15 seconds after which there was a 10 second gap before participants were required to relocate the objects to the correct location shown to them previously in an empty grid. There was no time limit for responding. This was repeated five times during the learning phase. For each of the five learning trials, a displacement score was calculated as the sum of the errors made for each object (calculated by counting the number of cells the object had to be moved both horizontally and vertically in order to be in the correct location). A total displacement score was calculated as the sum of the displacement scores on the five learning trials. A Learning Index was also calculated as the average relative difference in performance between trials (Kessels, Nys, Brands, van den Berg, & Van Zandvoort, 2006). The Learning Index equation can be visualised in Figure 2.2.

$$\frac{(A - B) / A + (B - C) / B + (C - D)/C + (D - E)/D}{4}$$

Figure 2.2 Learning Index equation where the letters A-E are indicative of the displacement scores for the five location learning trials respectively (Kessels et al., 2006).

2.2.4.2 Word presentation

Participants were presented sequentially with 15 words selected at random from a large bank of words derived from the MRC Psycholinguistic Database (Fearnley, 1997) and matched for word length, frequency, familiarity and concreteness. Stimulus duration was one second, with an inter-stimulus duration of one second.

2.2.4.3 Immediate word recall

Immediately after the word presentation, participants were given 60 seconds to write down as many of the 15 words that they were presented with during the stimulus presentation period. The task is scored for number of words correctly recalled.

2.2.4.4 Picture presentation

Participants were presented sequentially with 15 randomly selected photographic images to remember. Presentation was at a rate of one picture every three seconds, with a stimulus duration of one second.

2.2.4.5 Numeric working memory

A series of 5 digits from 1-9 were presented sequentially to the participant one at a time for them to memorise. Immediately following, 30 digits (15 targets and 15 distractors) were presented sequentially for the participant to indicate whether each digit was presented in the original series with a yes/no response. The task consists of 3 separate trials and outcomes are overall accuracy (% correct) and mean reaction time for correct responses (msec).

2.2.4.6 Choice reaction time

Arrows pointing either left or right appeared on the screen at irregular intervals ranging between 1 and 3 seconds. Participants were required to indicate the direction of the arrow as quickly as possible, whenever an arrow was displayed. Outcomes are accuracy (% correct) and reaction time for correct responses (msec).

2.2.4.7 Digit vigilance

A fixed digit ranging from 1-9 was presented on the right of the screen and a series of changing digits appeared on the left of the screen. Participants were required to respond with a single button press when both digits matched. Outcomes are accuracy (% correct), mean reaction time for correct responses (msec) and errors (number of false alarms).

2.2.4.8 Serial subtraction tasks

Computerised versions of the serial subtraction tasks were implemented using tests of 2-minute duration. Participants were required to count backwards in threes or sevens from a given number as quickly and as accurately as possible using the number keys to enter each response. A random starting number between 800 and 999 was presented on the computer screen, which is cleared by the entry of the first response. In the case of an incorrect response,

subsequent responses are scored as correct in relation to the previous number. The task is scored for total number of responses and the number of errors.

2.2.4.9 Rapid visual information processing (RVIP)

The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits were presented at the rate of 100 per minute and the participant responded to the detection of a target string by pressing the response button as quickly as possible. The task was continuous and lasted for 5 minutes, with 8 correct target strings presented in each minute. Outcomes are percentage of target strings correctly detected (% correct), average reaction time (msec) for correct detections, and number of false alarms.

2.2.4.10 Visual analogue scales (VAS)

Participants rated their current subjective ‘mental fatigue’ and ‘alertness’ states by placing an ‘X’ on a 100 mm line with the end points labelled “not at all” (left hand end; 0) and “extremely” (right hand end; 100).

2.2.4.11 Peg and ball

Two configurations were shown on the screen. In each, there was a blue, green and red ball each on one of 3 pegs. The configuration at the top of the screen was the goal configuration and participants were required to arrange the balls on the starting configuration (shown in the centre of the screen) to match the position of balls in the goal configuration. Participants were required to complete the task in the least number of moves possible with difficulty increasing as the task progressed. Outcomes were number of errors, average thinking time (msec) and average completion time (msec). Five stimuli at each of the three levels (3, 4 and 5 moves) were completed.

2.2.4.12 Stroop

In this computerised version of the classic task, words describing one of four colours (‘RED’, ‘YELLOW’, ‘GREEN’, ‘BLUE’) were presented in different coloured fonts in the centre of a computer screen. The participant was required to press one of four coloured response buttons in order to identify the font colour (e.g., if the word ‘GREEN’ was presented in a blue font, the

correct response would be to respond with the blue button). The presented words were either ‘congruent’ (word and font are the same colour) or ‘incongruent’ (word and font are different colours) and were presented in a random order. Outcomes are for accuracy (% correct) and mean reaction time for correct responses (msec).

2.2.4.13 Delayed word recall

Following completion of all other tasks, participants were given 60 seconds to write down as many of the 15 words that they were presented with during the stimulus presentation period described in section 2.2.4.2. The task is scored for number of words correctly recalled.

2.2.4.14 Delayed picture recognition

Thirty pictures comprising of the same 15 pictures from the stimuli presentation, described in section 2.2.4.4, plus 15 distractor pictures were presented, with participants making a yes/no response indicating whether the picture was in the original set. Outcomes are accuracy (% correct) and reaction time for correct responses (msec).

2.2.4.15 Delayed word recognition

Thirty words comprising the 15 words presented during the stimuli presentation (section 2.2.4.2) period plus 15 distractor words were presented, with the participant making a yes/no response indicating whether the word was in the original set. Outcomes are accuracy (% correct) and reaction time for correct responses (msec).

2.2.4.16 Computerised location learning recall

During the recall phase of the location learning task, which participants completed approximately 30 minutes after completion of the learning phase, participants were again asked to place the objects in the correction location on the empty grid as described in section 2.2.4.1 with no further prompting. The delayed trial was scored for displacement and a delayed displacement score was subsequently calculated as the difference between the displacement score on the final learning trial and the delayed trial.

2.2.5 Cognitive domain data

The thesis aimed to expand upon the findings of previous cross-sectional and intervention studies assessing cognitive performance in NAID women (Leonard et al., 2014; Murray-Kolb & Beard, 2007; Scott & Murray-Kolb, 2016) and therefore adopted the same paradigm as the intervention studies of assessing cognitive function by domain rather than by individual task outcomes to reduce the number of comparisons and for consistency throughout the thesis. Although it can be argued that reducing several cognitive outcomes to a single composite variable reduces the amount of information representing a single underlying construct (Schneider & Goldberg, 2020), cognitive composite measures are shown to improve the statistical power of analyses (Jacobs et al., 2020). Task assignment to cognitive domains was based upon a framework conceptualised in a literature review focussed upon the impact of breakfast consumption on cognitive function in adults (Galioto & Spitznagel, 2016) and by consulting alternate nutritional intervention studies that adopted a domain paradigm, which also used the same cognitive function assessment system (Haskell-Ramsay, Stuart, Okello, & Watson, 2017; Stonehouse et al., 2013). The chosen cognitive domains of attention, memory, learning and executive function were used throughout the thesis due to the cross-sectional differences in these domains identified by iron status and their amenability to iron supplementation across iron status groups (Cook et al., 2017; Falkingham et al., 2010; Greig et al., 2013; Lomagno et al., 2014; Scott et al., 2017; Scott & Murray-Kolb, 2016). The cognitive domains were confirmed by factor analyses using principal components analysis with varimax rotation; a component was retained if its eigenvalue was greater than 1.0 (See Appendix III). Ten cognitive domains were identified: attention accuracy, attention speed, episodic memory accuracy, episodic memory speed, executive function accuracy, executive function speed, learning accuracy, working memory accuracy, and working memory speed.

Cognitive domains were calculated by transforming individual task outcomes into standardised Z scores and grouping these scores into their respective cognitive domain, utilising the methodology previously advocated in nutritional intervention studies (Haskell-Ramsay et al., 2017; Stonehouse et al., 2013). Prior to calculating accuracy and speed scores, each task was required to be represented by one score so that all tasks were weighted equally within the domain. Consequently, for those tasks that had both correct and error outcomes (Digit Vigilance, RVIP and Serial Subtractions) an overall accuracy score was created by subtracting the standardised error score from their respective accuracy/total score and dividing the total by 2. For cLLT, the standardised total displacement score was subtracted from the respective delayed recall score and added to the standardised learning index score. This total was divided by 3 to create a total cLLT score. Scores were then re-standardised to create a final

standardised cLLT accuracy score. For the Peg and Ball task thinking time and completion time were averaged to create a total Peg and Ball reaction time score, which was then standardised. The specific calculations for each domain are outlined below.

2.2.5.1 Episodic memory

The episodic memory data consisted of episodic memory accuracy and speed. These two domain outcomes were calculated in a similar manner using standardised scores of task outcomes representative of the domain.

Episodic accuracy = (Zpicture recognition accuracy + Zword recognition accuracy + Zimmediate word recall correct + Zdelayed word recall correct) /4

Episodic speed = (Zpicture recognition RT + Zword recognition RT) /2

2.2.5.2 Working memory

The working memory data consisted of working memory accuracy and speed. The working memory accuracy score was calculated using standardised task outcomes including accounting for errors (serial subtractions) as described above:

Working memory accuracy = (Znumeric working memory accuracy + Zserial 3 subtractions accuracy + Zserial 7 subtractions accuracy) /3

The working memory speed score was calculated using the one standardised task outcome:

Working memory speed = (Znumeric working memory RT)

2.2.5.3 Executive function

The executive function data consisted of executive function accuracy and speed. The executive function accuracy score was calculated using standardised task outcomes representative of the domain:

Executive function accuracy = (Zstroop accuracy + ZCRT accuracy – Zpeg and ball errors) /3

The executive function speed score was calculated using standardised task outcomes accounting for multiple reaction time outcome measures (peg and ball) as described above:

Executive function speed = (Zstroop RT + ZCRT RT + Zpeg and ball RT)

2.2.5.4 Attention

The attention data consisted of attention accuracy and speed. The attention accuracy score was calculated using standardised task outcomes accounting for errors (RVIP and digit vigilance) as previously described:

Sustained attention accuracy = (ZRVIP accuracy + Zdigit vigilance accuracy) /2

The attention speed score was calculated from standardised scores using the following formula:

Sustained attention speed = (ZRVIP RT + Zdigit vigilance RT) /3

2.2.5.5 Learning

Learning comprised of the cLLT task, domain score was calculated as described in section 2.2.5.

cLLT = (ZDelayed Recall – ZTotal Displacement + ZLearning Index) /3

2.2.6 Behavioural function assessments

2.2.6.1 Profile of mood states (POMS) (McNair, 1992)

The Profile of mood states (POMS) questionnaire comprises 65 adjectives rated on a 5-point scale of 0 (not at all) to 4 (extremely) for how the participant is feeling right now. Each item is consolidated into the sub-scales of tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment. A total ‘mood disturbance’ score (TMD) is obtained by subtracting the vigour-activity score from the sum of tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia and confusion-bewilderment scores. For TMD, higher scores are indicative of greater mood disturbance. The POMS is widely used and

demonstrates internal consistency across subscales (α 0.63-0.96) (Curran, Andrykowski, & Studts, 1995).

2.2.6.2 Piper fatigue scale (PFS) (Piper et al., 1998)

The Piper fatigue scale (PFS) is a 22-item questionnaire assessing subjective feelings of fatigue. Questions are answered on a 1-10 scale. Each item is consolidated into the subscales of behavioural/severity, affective meaning, sensory, and cognitive/mood. A total fatigue score is computed as the average of all responses; higher scores are indicative of greater subjective fatigue. The PFS is widely used and validated in clinical populations in several languages (Cantarero-Villanueva et al., 2014; Jang, Kim, & Lee, 2017; Lundgren-Nilsson, Dencker, Jakobsson, Taft, & Tennant, 2014; Reeve et al., 2012) and has been validated within non-clinical populations (Clark, Ashford, Burt, Aycock, & Kimble, 2006).

2.2.6.3 Sleep condition indicator (SCI) (Espie et al., 2014)

The Sleep condition indicator (SCI) is an 8-item questionnaire assessing sleep quality over the last month. Questions are answered on a 5-point scale (0-4) and a total SCI score is calculated by summing across all scale items. Total scores can range from 0 to 32; higher total scores are indicative of better sleep quality. The SCI also has a cut-off point for detecting clinically significant insomnia (scores ≤16). The SCI is a reliable measure of sleep quality (α ≥.86) and has shown convergent validity with the validated Pittsburgh Sleep Quality Index and Insomnia Severity Index (Espie et al., 2014).

2.2.6.4 Perceived stress scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

The Perceived stress scale (PSS) is a 10-item questionnaire assessing feelings and thoughts surrounding situations one may deem as stressful having occurred during the last month. Questions are answered on a 5-point scale of 0 (never) to 4 (very often) and a total PSS score is obtained by reversing responses to the four positively worded items (4, 5, 7 & 8) and then summing across all scale items. Total scores can range from 0 to 40; a higher total PSS score is indicative of higher perceived stress. The PSS is a widely used research questionnaire, which has established acceptable psychometric properties amongst diverse populations (Lee, 2012).

2.2.6.5 Health outcomes SF-12 (Ware, Kosinski, & Keller, 1996)

The SF-12 is a 12-item questionnaire used as a measure of generic health and well-being outcomes. The items consolidate into eight health domain outcome scales: physical functioning, role limitations due to physical health problems (role-physical), bodily pain, general health, vitality, social functioning, role limitations due to emotional problems (role-emotional) and mental health. For physical functioning, role-physical, general health, vitality, social functioning, role-emotional and mental health domain scores, low scores are indicative of health disturbance whilst higher scores indicate little to no effect on health. Similarly, for bodily pain scores, lower scores are indicative of greater levels of pain affecting normal activities whilst higher scores indicate that pain does not affect activity. Two component summary measures are calculated by combining scores on the four relevant outcome scales; a 'physical component' summary (PCS) using physical functioning, role-physical, bodily pain and general health scores; a 'mental component' summary (MCS) using vitality, social functioning, role-emotional and mental health scores. Scores are calculated using the QualityMetric Health Outcomes™ Scoring System to accommodate norm-based standardisation of scale scores. Each health domain scale and component summary score have means of 50 and a standard deviation of 10 based upon the 2009 United States general population sample (Kosinski, Ware, Turner-Bowker, & Gandek, 2007). Therefore, scores greater or lesser than 50 are indicative of scores above or below the average, respectively. The SF-12 is a widely used alternative for the SF-36 Health Outcomes questionnaire and has been validated for use in several European countries (Gandek et al., 1998).

2.2.6.6 NASA task load index (NASA-TLX) (Hart, 2006; Hart & Staveland, 1988)

The NASA-TLX tool is a subjective workload assessment comprising 6-items. With permission, the standard pen and paper version was adapted for use on COMPASS through the use of a VAS. Following completion of the cognitive task battery, participants rated their subjective feelings of mental demand, physical demand, temporal demand, performance, effort and frustration. All scales were answered by placing an 'X' on a 100 mm line with the end points labelled "very low" (left hand end; 0) and "very high" (right hand end; 100) with the exception of the performance scale where end points were "perfect" (left hand; 0) and "failure" (right hand; 100). A total workload score following cognitive assessment is calculated using the average of all responses; higher scores are indicative of greater subjective workload. As advised, physical demand scores were not included in the total workload calculations due to a lack of relevance to the cognitive tasks employed.

The cognitive and behavioural assessments described in sections 2.2.4 and 2.2.6, respectively, are depicted in Figure 2.3 in order of presentation to participants.

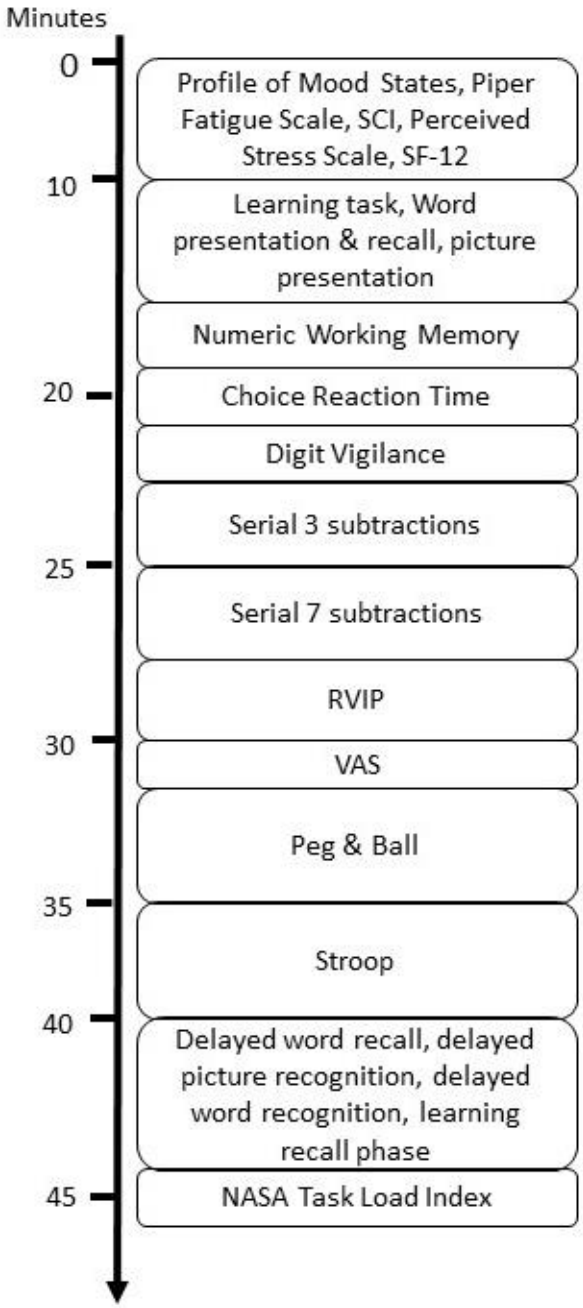


Figure 2.3 Schematic of the cognitive and mood assessments completed during study testing. SCI, sleep condition indicator; RVIP, Rapid visual information processing; VAS, visual analogue scales.

2.2.7 Blood sampling and analysis

For all studies throughout the thesis, blood samples collected by venepuncture and finger-prick were necessary for participant enrolment and iron status determination. For iron

intervention studies, it is recommended to use haemoglobin and serum ferritin for accurate iron status determination (Joint World Health Organization/Centers for Disease & Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, 2007). However, as serum ferritin is an acute phase reactant, it is recommended to also measure markers of inflammation such as CRP (Namaste et al., 2017).

2.2.7.1 Haemoglobin

The finger-prick blood sample was used for the measurement of haemoglobin with the mobile HemoCue® Hb 201+ Analyser. The HemoCue® test method has shown good reproducibility for determining haemoglobin concentrations in capillary blood samples (da Silva Pereira et al., 2020) and good within- and between-model reproducibility (Lin's concordance correlation coefficient > 0.90 in all cases) for Hb201+ and Hb 301 instruments (Jain, Chowdhury & Jain, 2018). Standardised capillary blood collection methods were followed; after sterilising the participant's middle finger of their non-dominant hand, light pressure was applied towards the fingertip and a lancet was used to make a puncture. The first 2-3 drops of blood were wiped away with sterile gauze before collecting a large enough blood sample to fill the microcuvette in one continuous process. Excess blood was wiped from the microcuvette before placing into the analyser tray, which displayed the haemoglobin value in g/L. A cut-off of <120 g/L was used throughout the thesis to determine anaemia in accordance with worldwide guidelines (World Health Organization, 2011a).

2.2.7.2 Serum ferritin

For serum ferritin, blood samples were obtained via venepuncture into serum separator tube (SST) vacutainers (8.5 ml). Samples were inverted 6 times, refrigerated at 5 °C, and allowed to clot for at least 30 minutes before they could be processed within 2 hours of acquisition. Samples were centrifuged at 3000 rpm (x 1734 g) for 10 minutes at 4 °C. Following centrifugation, 1 ml of serum was transferred into two 1.5 ml Eppendorf tubes® and immediately frozen at -80 ^{0}C prior to analysis conducted by Newcastle Laboratories, Newcastle-upon-Tyne.

Samples were analysed using the Roche ECLIA® electrochemiluminescence two-site sandwich immunoassay technology. The serum samples were aliquoted into false-bottomed tubes following receipt at the laboratory. During the first incubation of the serum samples, ferritin was combined with a biotinylated monoclonal ferritin-specific antibody and a

monoclonal ferritin-specific antibody labelled with a ruthenium complex to form a sandwich complex. During the second incubation following addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were removed using Procell (Roche Diagnostics GmbH). A voltage was then applied to the electrode to induce chemiluminescent emission for measurement by a photomultiplier. Results were determined by measuring the electrochemiluminescence signal obtained from the reaction product of the sample against a calibration curve generated by a 2-point calibration and a master curve provided via the reagent barcode. This method of serum ferritin analysis has been assessed for within-run (4.8±2.3%; CI 3.88–5.72%) and between-run imprecision (6.9±2.6%; CI 5.70–8.14%); these values are comparable to alternate laboratory methods, which is indicative of comparable accuracy and performance across methods (Garcia-Casal et al., 2018). Final serum ferritin results were provided in µg/L.

A cut-off of 20 µg/L was used for ID in line with previous nutritional intervention studies (Leonard et al., 2014) and evidence suggesting that a cut off of 30 µg/L is subject to risk of false-positives (Daru et al., 2017), whilst depletion of iron stores is reflected by levels between 13-20 µg/L (Langley-Evans, 2013).

2.2.7.3 C-reactive protein (CRP)[1]

For CRP, blood samples were obtained via venepuncture into serum separator tube (SST) vacutainers (8.5 ml). Samples were inverted 6 times, refrigerated at 5 °C, and allowed to clot for at least 30 minutes before they could be processed within 2 hours of acquisition. Samples were centrifuged at 3000 rpm (x 1734 g) for 10 minutes at 4 °C. Following centrifugation, 1 ml of serum was transferred into two 1.5 ml Eppendorf tubes® and immediately frozen at -80 ^{0}C prior to analysis conducted by the Newcastle Laboratories, Newcastle-upon-Tyne.

Samples were analysed by particle-enhanced immunoturbidimetric assay. CRP in the sample reacts specifically with the anti-human CRP antibodies coated on the latex particles to yield

[1] Serum samples were analysed for CRP after completion of data collection. Participants with CRP concentrations >10 mg/L were excluded from the per protocol population.

insoluble aggregates. The turbidometric absorbance of the aggregates is proportional to the CRP concentration in the samples. A serum CRP threshold of 3-10 mg/L is considered normal when interpreting micronutrient status (Joint World Health Organization/Centers for Disease & Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, 2007) whilst CRP concentrations >10 mg/L are indicative of marked elevation and are thus considered positive for infection, inflammation or tissue injury (Nehring, Goyal, Bansal, & Patel, 2020).

2.2.7.4 Iron status parameters

Upon gaining individual haemoglobin and serum ferritin concentrations, participants were assigned to one of four iron status groups; iron sufficient (haemoglobin ≥120 g/L and serum ferritin >20 µg/L), non-anaemic iron deficient (haemoglobin ≥120 g/L and serum ferritin ≤20 µg/L); iron deficient anaemic (haemoglobin <120 g/L and serum ferritin ≤20 µg/L) or anaemic without ID (haemoglobin <120 g/L and serum ferritin >20 µg/L).

2.2.8 Procedure

All study visits took place at Northumbria University's Brain, Performance and Nutrition Research Centre (BPNRC). Potential participants were initially screened by the principal investigator over telephone or email to determine whether they were eligible to attend a full screening appointment. During this initial screening, menstrual cycle information was gathered so that those who had a regular menstrual cycle or withdrawal bleed attended the laboratory during the last week of their menstrual cycle's luteal phase (approximately day 21-28 or equivalent), consistent with when haemoglobin and serum ferritin should be at their highest (Kim et al., 1993). Those using contraceptive methods that halted bleeds attended based on their and the researcher's availability.

Following successful screening, potential participants were invited for a full screening visit on site. The requirements of the study were discussed in line with the participant information sheet supplied to the participant prior to screening. Following written informed consent, demographic data was collected, and participants underwent a health screening and provided finger-prick and venous blood samples. The blood samples were used to determine haemoglobin, serum ferritin and CRP. CRP was measured as an inflammation-correction approach to account for falsely elevated serum ferritin due to infection and inflammation

(Suchdev et al., 2017). Participants were also required to complete lifestyle questionnaires regarding daily caffeine consumption, physical activity, food frequency intake and menstrual blood loss.

Training on the computerised cognitive tasks was then completed to further confirm eligibility. The training session followed standard operating procedures designed to decrease the chance of learning effects during the main trials. Participants were required to complete three shortened versions of each task to gain familiarity, followed by two full-length versions. Following the shortened tasks, participant's scores were compared to the shortened task norms for the researcher to provide performance feedback. This enabled participants to perform at their best during one of the full-length versions. Extra training was provided where necessary, however once participants completed the session to the required standards, they were eligible to be enrolled into the study.

Before attending the testing session, participants were asked to fast for 12 hours, avoiding intake of all food and drink except water. Participants were also asked to avoid alcohol and the intake of 'over the counter' medication for 24 hours. Participants with natural or withdrawal bleeds were required to return for this session 7-14 days following bleed onset; due to menstrual cycle phase impacts on brain activation (Pletzer, Harris, Scheuringer, & Hidalgo-Lopez, 2019) and emotion-related changes exhibited in the luteal phase and menses (Sundstrom Poromaa & Gingnell, 2014). Those without bleeds were required to attend approximately two weeks following their initial appointment to allow time for blood sample analysis. Upon confirmation of continued eligibility, participants completed the cognitive and mood assessments outlined in Figure 2.3, which took approximately forty-five minutes. Once all assessments were completed, participants were fully debriefed and were given their haemoglobin and serum ferritin results. Participant's iron status was only disclosed to them following completion of the cognitive and mood assessments to avoid the potential influence this may have had on responses.

2.2.9 Data cleaning

Prior to conducting analyses, procedural deviations were checked to identify the per protocol population. The data were then investigated for anomalous and outlier data. Initially, raw data were inspected for any 0 values on cognitive assessments, which would be indicative of a failure to respond to task stimuli. All analyses were conducted using SPSS (Version 26) and boxplots were generated for individual variables to identify potential outliers. The boxplots visually depict the numerical data and skewness; the box represents the middle 50 % of values

and the whiskers depict the upper and lower 25 % of values. Five sample statistics (the minimum score, lower quartile, median, upper quartile and maximum score) provide additional information regarding the numerical distribution of data. Outliers beyond the minimum and maximum scores that are more than one and a half box lengths from either end of the box are denoted by a circle. Those beyond three box lengths from either end of the box are denoted with an asterisk to indicate an extreme outlier. Outliers marked with an asterisk were removed whilst those marked with a circle were removed if they were also noted as deviating from the normal distribution following inspection of residual plots and histograms. Any additional values identified as deviating from the normal distribution were also removed. Upon completion of these data cleaning procedures for each outcome variable, data analysis commenced.

2.2.10 Statistical methods

Data were analysed using regression models in SPSS (Version 26). The analysis was conducted in three phases to address the outcomes of interest. Initially, data were analysed using an enter method multiple regression. This was used to determine the contribution of age, physical activity, BMI, dietary iron and menstrual blood loss to predict iron status, haemoglobin and serum ferritin. Enter method multiple regression was then used to determine the extent to which age, physical activity, BMI, dietary iron and menstrual blood loss predict cognitive performance, subjective mood, fatigue, health and well-being. Years in education was also included as a predictor variable for the cognitive performance and workload regression models (Gallego, Barcos, Correa, Sánchez Espinosa, & Callejo, 2016; Lövdén, Fratiglioni, Glymour, Lindenberger, & Tucker-Drob, 2020; Weber & Skirbekk, 2014). If any of the demographic/lifestyle variables were found to have significantly predicted any of the outcome variables comprising cognitive performance, subjective mood, fatigue, health or well-being, they were then included in an adjusted regression model alongside iron status or haemoglobin and ferritin. This was first used to determine whether iron status as a categorical factor could predict cognitive performance, subjective mood, fatigue, health or well-being whilst controlling for these variables. Secondly, it was used to determine whether haemoglobin and serum ferritin as continuous variables could predict cognitive performance, subjective mood, fatigue, health or well-being whilst controlling for these variables. If none of the demographic/lifestyle variables were able to significantly predict the outcome variables in the enter method regression models, an unadjusted multiple regression model was used.

2.3 Results

2.3.1 Participants

Of the 414 participants screened 379 were enrolled into the study. Eighteen participants were lost to follow-up and one participant was excluded as their serum ferritin sample had haemolysed meaning no reading could be obtained for iron status determination; 361 participants subsequently completed the study. As the focus of the thesis is upon NAID, participants categorised as IDA (N = 38) and anaemic without ID (AWID) (N = 59) were excluded from the analyses. The per protocol analysis excluded thirteen participants for not complying with day of menstrual cycle requirements for either their screening or testing visit; four for elevated serum ferritin in the absence of inflammation (> 150 µg/L confers a risk of iron overload in the general population (World Health Organization, 2011b); four for haemoglobin outside of the normal range of 120-150 g/L (Lewis, Osei-Bimpong, & Bradshaw, 2004); four for concomitant medication; three for elevated CRP (> 10 mg/L) and one for missing cognitive data. The final sample for data analysis was N = 235; 162 participants were IS (haemoglobin ≥120 g/L and serum ferritin >20 µg/L) and 73 were NAID (haemoglobin ≥120 g/L and serum ferritin ≤20 µg/L). Participant disposition through the study can be found in Figure 2.4 and their demographic data is displayed in Table 2.1 by iron status group.

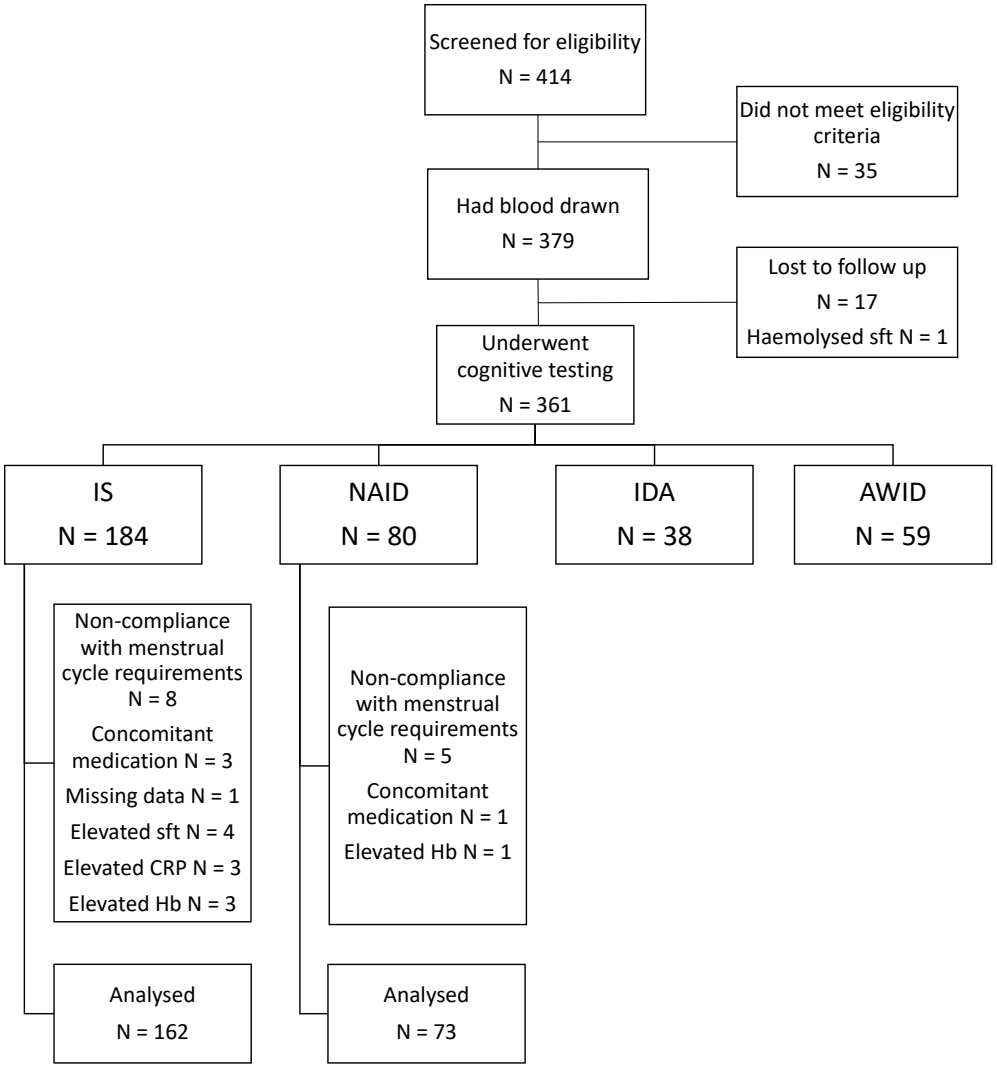


Figure 2.4 Participant disposition through the trial, culminating in N = 235 of the 361 whom underwent testing. IS = iron sufficient; NAID = non-anaemic iron deficient; IDA = iron deficient anaemic; AWID = anaemia without iron deficiency; sft = serum ferritin; CRP = C-reactive protein; Hb = haemoglobin

Table 2.1 Participant demographic information and characteristics. Means and Std. Deviation (SD) are presented with F and p values of the main effects from the one-way ANOVAs conducted on the baseline data by iron status group.

		N	Baseline		Main effects	
			Mean	SD	*F*	*P*
Haemoglobin (g/L)	**NAID**	**73**	**126.15**	**6.32**	**5.47**	**.020**
	IS	162	128.29	6.56		
Serum ferritin (µg/L)	**NAID**	**73**	**14.53**	**4.57**	**143.78**	**<.001**
	IS	**162**	**56.62**	**29.80**		
Age (years)	NAID	73	25.92	7.88	3.33	.069
	IS	162	28.15	9.03		
Years in education	NAID	72	16.58	2.63	.535	.465
	IS	162	16.84	2.40		
BMI (kg/m^2)	NAID	73	23.99	3.72	1.69	.195
	IS	162	24.70	3.95		
Systolic BP (mmHg)	NAID	73	116.77	9.27	.017	.895
	IS	161	116.57	11.53		
Diastolic BP (mmHg)	NAID	73	77.30	6.72	1.36	.245
	IS	161	78.60	8.36		
Menstrual blood loss score	NAID	55	3.73	2.50	1.76	.186
	IS	115	3.21	2.32		
Physical activity (MET minutes/week)	**NAID**	**71**	**3674.70***	**2297.67**	**6.07**	**.015**
	IS	**158**	**2939.88***	**1962.16**		
Cereals and cereal products (g/day)	NAID	72	240.25	91.78	.014	.905
	IS	160	238.21	131.27		
Dietary iron (mg/day)	NAID	72	10.67	3.58	.050	.824
	IS	160	10.79	3.59		
Fish and fish products (g/day)	NAID	72	33.83	31.90	.690	.407
	IS	160	40.33	62.82		
Meat and meat products (g/day)	NAID	72	92.08	72.65	2.56	.111

	IS	160	109.85	80.69		
Nuts and seeds (g/day)	NAID	72	12.79	23.88	.593	.442
	IS	160	10.32	21.97		
Vegetables (g/day)	NAID	72	306.04	155.60	.349	.555
	IS	160	321.68	198.79		
Alcohol consumption (units/day)	NAID	73	0.78	0.85	1.76	.186
	IS	162	0.99	1.18		
Caffeine consumption (mg/day)	NAID	71	199.18	141.08	.061	.805
	IS	160	204.52	155.74		

* = significant difference between iron status groups below $p < .05$.

Due to the number of statistical analyses performed, only those which revealed significant models are reported throughout. Data are reported in full in Appendix IV.

2.3.2 Demographic/lifestyle factor predictors of different measures of iron status, cognitive function, subjective mood, fatigue, health and well-being

2.3.2.1 Iron status groups

No significant contribution of the predictor variables was identified for iron status.

2.3.2.2 Haemoglobin

No significant contribution of the predictor variables was identified for haemoglobin.

2.3.2.3 Serum ferritin

A significant model was identified that accounted for 7% of the variance in serum [$R^2 = .068$, F (5, 157) = 2.3, $p = .047$]. Menstrual blood loss scores made a significant negative contribution to the regression [$\beta = -.165$; t (157) = -2.11, $p = .036$].

Demographic/lifestyle predictors of cognitive function, subjective mood, fatigue and wellbeing are presented in Table 2.3.

Table 2.2 Demographic/lifestyle factor predictors of cognitive function, subjective mood, fatigue, health and well-being. R^2 and β-weightings are presented from the models of best fit. All values are significant to $p < .05$ unless indicated.

Psychological measures		Predictor	R^2	β	$Partial^2$
Cognitive function	Working Memory Accuracy	Years in education	.067	.201	.039
	Executive Function Speed	Age	.079	.237	.050
	Attention Accuracy	Age	.068	.227	.046
	Learning	BMI	.061	-.174	-.028
Subjective mood (PSS; SCI)	Perceived Stress	Menstrual blood loss	.066	.164	.027
	Sleep Quality	Menstrual blood loss	.100*	-.274	-.073
Subjective fatigue (PFS; VAS)	Behavioural-Severity	Menstrual blood loss	.042	.165	.026
	Affective-Meaning	Menstrual blood loss	.057	.179	.031
	Alertness	Menstrual blood loss	.067	-.168	.028
Subjective wellbeing (SF12)	Vitality	BMI	.075	-.189	-.034
		Physical activity	.075	.163	.027

* $p < .05$

2.3.3 Iron status and associations with cognitive function, subjective mood, fatigue, health and well-being

2.3.3.1 Cognitive domains

Neither the unadjusted nor adjusted models revealed any significant impact of iron status for the cognitive domains (Table 2.4).

Table 2.3 Cognitive task analysis outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[2,3]

		N					
			Mean	SE	R^2	*β*	*p*
Episodic Accuracy	NAID	73	0.02	0.08	.000	-.017	.801
	IS	162	-0.01	0.06			
Episodic Speed	NAID	73	0.04	0.11	.001	-.023	.726
	IS	160	-0.00	0.07			
Working Memory Accuracy	NAID	73	0.07	0.10	.043	-.097	.134
	IS	162	-0.08	0.06			
Working Memory Speed	NAID	72	-0.11	0.11	.005	.072	.273
	IS	159	0.05	0.08			
Executive Function Accuracy	NAID	73	-0.05	0.07	.002	.047	.472
	IS	162	0.02	0.05			
Executive Function Speed	NAID	73	-0.04	0.09	.061	.014	.823
	IS	162	0.02	0.06			
Sustained Attention Accuracy	NAID	73	0.04	0.10	.048	-.089	.170
	IS	162	-0.08	0.07			
Sustained Attention Speed	NAID	73	0.01	0.11	.000	.019	.769
	IS	162	0.04	0.06			
Learning	NAID	72	0.02	0.09	.033	.000	.995
	IS	158	-0.01	0.06			

[2] Unadjusted models are presented for analysis outcomes that did not have any significant demographic/lifestyle predictors. Adjusted models are presented for analysis outcomes that did have significant demographic/lifestyle predictors from Table 2.3.

[3] For accuracy outcomes, positive values are indicative of better performance and negative values of worse performance. For speed outcomes, positive values are indicative of slower speed and positive values of slower speed.

2.3.3.2 Subjective mood

2.3.3.2.1 POMS

Neither the unadjusted nor adjusted models revealed any significant impact of iron status for the POMS subscales (Table 2.5).

Table 2.4 Subjective mood analysis (POMS) outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[4]

		N					
			Mean	SE	R^2	*β*	*P*
Tension-Anxiety	NAID	72	6.64	0.57	.001	-.030	.646
	IS	158	6.32	0.39			
Depression-Dejection	NAID	68	2.02	0.36	.000	-.008	.911
	IS	151	1.97	0.24			
Anger-Hostility	NAID	68	1.37	0.24	.002	.041	.543
	IS	150	1.55	0.16			
Vigour-Activity	NAID	72	13.01	0.74	.000	-.008	.898
	IS	159	12.90	0.50			
Fatigue-Inertia	NAID	72	7.04	0.61	.000	.011	.868
	IS	159	7.16	0.41			
Confusion-Bewilderment	NAID	72	9.18	0.60	.000	-.019	.777
	IS	159	8.98	0.40			
Total Mood Disturbance	NAID	65	9.29	2.14	.000	.016	.812
	IS	145	10.76	1.43			

[4] Unadjusted models are presented for analysis outcomes that did not have any significant demographic/lifestyle predictors. Adjusted models are presented for analysis outcomes that did have significant demographic/lifestyle predictors from Table 2.3.

2.3.3.2.2 PSS and SCI

Neither the unadjusted nor adjusted models revealed any significant impact of iron status for ratings of perceived stress or sleep quality (Table 2.6).

Table 2.5 Subjective mood analysis (PSS and SCI) outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[5]

		N	Mean	SE	R^2	*β*	*P*
Perceived Stress	NAID	55	19.25	0.92	.034	-.059	.439
	IS	115	17.38	0.64			
Sleep Quality	NAID	55	19.95	0.93	.080	.051	.491
	IS	115	21.91	0.64			

2.3.3.2.3 NASA-TLX

No significant confounding variables were identified to impact ratings of total workload. The unadjusted model revealed no significant impact of iron status for ratings of total workload (Table 2.7).

Table 2.6 Subjective mood analysis (NASA-TLX) outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[6]

		N	Mean	SE	R^2	*β*	*P*
Total Workload	NAID	72	53.10	1.45	.015	.123	.064
	IS	158	56.35	0.98			

[5,6] Unadjusted models are presented for analysis outcomes that did not have any significant demographic/lifestyle predictors. Adjusted models are presented for analysis outcomes that did have significant demographic/lifestyle predictors from Table 2.3.

2.3.3.3 Subjective fatigue (PFS and VAS)

Neither the unadjusted nor adjusted models revealed any significant impact of iron status for ratings of subjective fatigue (Table 2.8).

Table 2.7 Subjective fatigue analysis (PFS and VAS) outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[7]

		N					
			Mean	SE	R^2	*F*	*P*
Behavioural-Severity	NAID	55	3.66	0.27	.032	-.031	.689
	IS	115	3.31	0.18			
Affective Meaning	NAID	55	4.83	0.27	.038	-.051	.503
	IS	115	4.78	0.19			
Sensory	NAID	73	5.60	0.23	.004	-.060	.361
	IS	162	5.35	0.15			
Cognition-Mood	NAID	73	4.57	0.20	.000	-.003	.964
	IS	162	4.56	0.13			
Total Fatigue	NAID	55	4.64	0.23	.003	-.051	.437
	IS	115	4.48	0.16			
Mental Fatigue	NAID	73	59.60	2.11	.004	.062	.343
	IS	162	62.02	1.42			
Alertness	NAID	54	41.50	2.55	.030	-.070	.364
	IS	115	38.85	1.74			

2.3.3.4 Subjective wellbeing (SF-12)

[7] Unadjusted models are presented for analysis outcomes that did not have any significant demographic/lifestyle predictors. Adjusted models are presented for analysis outcomes that did have significant demographic/lifestyle predictors from Table 2.3.

Neither the unadjusted nor adjusted models revealed any significant impact of iron status for the SF-12 scales (Table 2.9).

Table 2.8 Subjective wellbeing analysis (SF-12) outcomes for NAID and IS groups. Means and standard error (SE) are presented with R^2, β-weightings and p values of the unadjusted or adjusted models for iron status[8]

		N					
			Mean	SE	R^2	*F*	*P*
Physical Functioning	NAID	73	54.59	0.73	.007	-.085	.193
	IS	161	53.54	0.49			
Role-Physical	NAID	73	51.72	0.82	.000	.021	.745
	IS	161	52.05	0.56			
Bodily Pain	NAID	73	54.15	0.81	.005	-.072	.274
	IS	161	53.08	0.54			
General Health	NAID	73	52.19	0.98	.001	.026	.690
	IS	160	52.66	0.66			
Vitality	NAID	70	49.33	0.98	.068	.074	.266
	IS	153	50.75	0.66			
Social Functioning	NAID	72	47.88	0.91	.010	.101	.126
	IS	159	49.57	0.62			
Role-emotional	NAID	73	42.33	1.21	.006	.077	.238
	IS	161	44.05	0.81			
Mental health	NAID	73	42.76	1.06	.014	.117	.074
	IS	160	45.07	0.72			
PCS	NAID	72	57.86	0.66	.013	-.112	.091
	IS	157	56.51	0.45			
MCS	NAID	71	41.54	1.13	.013	.115	.083
	IS	158	43.91	0.76			

[8] Unadjusted models are presented for analysis outcomes that did not have any significant demographic/lifestyle predictors. Adjusted models are presented for analysis outcomes that did have significant demographic/lifestyle predictors from Table 2.3.

2.3.4 Haemoglobin and serum ferritin associations with cognitive domains, subjective mood, fatigue, health and well-being

2.3.4.1 Cognitive domains

Neither the unadjusted nor adjusted models revealed any significant impact of haemoglobin nor serum ferritin for the cognitive domain outcomes.

2.3.4.2 Subjective mood

2.3.4.2.1 POMS

Neither the unadjusted nor adjusted models revealed any significant impact of haemoglobin nor serum ferritin for the POMS subscales.

2.3.4.2.2 PSS and SCI

Neither the unadjusted nor adjusted models revealed any significant impact of haemoglobin nor serum ferritin for ratings of perceived stress or sleep quality.

2.3.4.2.3 NASA-TLX

No significant confounding variables were identified to impact ratings of total workload. The unadjusted model revealed no significant impact of haemoglobin nor serum ferritin for ratings of total workload.

2.3.4.3 Subjective fatigue (PFS)

Neither the unadjusted or adjusted models revealed any significant impact of haemoglobin nor serum ferritin for ratings of subjective fatigue.

2.3.4.4 Subjective well-being (SF-12)

For PCS and its components, a significant model was only identified for bodily pain. No confounding variables were identified to impact ratings of bodily pain. The unadjusted model significantly accounted for 2.6 % of the variance in bodily pain scores [R^2 = .026, F (2, 231) =

3.06, p = .049]. However, neither haemoglobin (p = .099) nor serum ferritin significantly contributed to the regression (p = .178).

A significant model was identified for MCS, for which no confounding variables were identified. The unadjusted model significantly accounted for 2.7 % of the variance in MCS [R^2 = .027, F (2, 226) = 3.09, p = .047]. Serum ferritin made a significant positive contribution to the regression [β = .169, t (226) = 2.48, p = .014]. Haemoglobin did not significantly contribute to the regression (p = .621).

Within MCS, role limitations due to emotional functioning was the only component for which a significant model was revealed. No confounding variables were identified to impact role limitations due to emotional functioning. The unadjusted model significantly accounted for 3.4 % of the variance in emotional functioning scores [R^2 = .034, F (2, 231) = 4.10, p = .018]. Serum ferritin made a significant positive contribution to the regression [β = .181, t (231) = 2.70, p = .008]. Haemoglobin did not significantly contribute to the regression (p = .105).

2.4 Discussion

Overall, the results from the current study revealed no significant associations between psychological function and categorical iron status. Higher serum ferritin concentrations were associated with better emotional functioning and higher mental component summary scores, indicating better mental functioning.

With regards to serum ferritin, significant positive associations were identified with emotional functioning and MCS scores as indicators of health-related quality of life and well-being. Current knowledge surrounding the impact of iron status upon health and well-being is mixed as NAID women of reproductive age have previously exhibited significantly lower MCS scores than their IS counterparts (Patterson, Brown, & Roberts, 2001), yet further investigations failed to identify any significant differences in perceived health and well-being between NAID and IS women (Beck et al., 2012). In comparison, chi-square analysis revealed that NAID women were significantly more likely than their IS counterparts to score below average on MCS in the current study, and a specific role of serum ferritin in emotional functioning and mental health has been identified. A potential explanation for this may surround the dependence of monoamine synthesis, including dopamine, serotonin and noradrenaline, upon non-haem iron (Kim & Wessling-Resnick, 2014); a reduction in serum ferritin and thus reduced stored iron may cause emotional dysregulation and negative affectivity through feelings of depression (Vahdat Shariatpanaahi et al., 2007), anxiety (Lozoff et al., 2000), fatigue (Meeusen et al., 2006) and aggression (Chester et al., 2016) that can profoundly affect individuals' mental health and wellbeing (Ferreira et al., 2019). Although no associations were identified for these individual mood states in the current study, their combination may contribute to an overall representation of emotional functioning and mental health-related quality of life. Interpretation of such findings should however err on the side of caution as the contribution of serum ferritin for explaining indicators of health-related quality of life and well-being was very small.

The current study identified no significant associations between cognitive function and categorical iron status, consistent with previous findings (Cook et al., 2017; Leonard et al., 2014; Murray-Kolb & Beard, 2007; Scott & Murray-Kolb, 2016). When haematological parameters were treated as continuous variables, no significant associations with cognition were achieved in contrast to earlier findings (Blanton, 2013; Blanton et al., 2013; Scott & Murray-Kolb, 2016). Overall, these findings are suggestive of cognition not declining in the early stages of ID. It must be considered however that compensatory neural adaptations may be responsible for such findings. To meet the metabolic demands of increased neural activity

under normal conditions, cerebral blood flow (CBF) is augmented to supply the metabolic resources required to fulfil the demand for tissue oxygen and glucose (Kozberg & Hillman, 2016; Vazquez, Masamoto, Fukuda, & Kim, 2010). As NAID is shown to impair cardiomyocyte contractility (Hoes et al., 2018), blood viscosity is subsequently increased (Broberg et al., 2006; Khaled et al., 1998). To compensate for this action, vasodilation is increased, and it is only when this action is exhausted that CBF is impaired. Further investigation is thus warranted using neuroimaging techniques to determine whether there are significant differences in CBF between iron status groups.

The null findings concerning the mood, fatigue, stress and sleep quality outcomes are suggestive of no behavioural differences between NAID and IS women of reproductive age. This contributes to the largely equivocal literature as although NAID is shown to be 15 % greater in women with depression compared to those without (Vahdat Shariatpanaahi et al., 2007), independent associations between iron status parameters of haemoglobin and serum ferritin and depressive symptoms only present with a concomitant presentation of fatigue (Price et al., 2017). Though meta-analysis revealed that significant associations between NAID and fatigue were only identified in populations who reported with a primary concern of fatigue and not within the general population (Yokoi & Konomi, 2017), as in the current study. Further cross-sectional investigations are required to determine whether associations between NAID and behavioural function exist within the general population of women of reproductive age whilst taking the role of fatigue into account.

The current study has highlighted the increased prevalence of NAID compared to IDA (22 % v 11.4 %, respectively) in a general population of women of reproductive age based in the United Kingdom, which is consistent with previous international epidemiological data (Soppi, 2018; Umbreit, 2005). As the relationship between IDA and cognitive and behavioural dysfunction is already well-established (Jáuregui-Lobera, 2014) and the AWID group suggests anaemia to be independent of iron, excluding these two groups enabled an analysis and interpretation focussed specifically upon discovering the effects associated with the more prevalent NAID compared to their IS counterparts. Additionally, several limitations concerning previous cross-sectional investigations have been addressed including a thorough assessment of iron status by controlling for inflammation, categorising iron status based upon a more diagnostically accurate serum ferritin cut-off and considering the potentially confounding effects of several key variables. Analyses were adjusted to comprehensively account for variables with an established relationship to the cognition, mood, fatigue, and wellbeing outcomes to ensure the most accurate determination of the influence of iron status, haemoglobin, and serum ferritin on psychological function in women of reproductive age.

However, it is interesting to note that dietary iron did not significantly contribute to any of the haematological, cognitive, or behavioural outcomes. When compared to menstrual blood loss, which significantly contributed to serum ferritin concentrations and multiple mood and fatigue outcomes, the current study provides evidence to suggest that it may be more important to correct heavy menstrual blood loss than to increase dietary iron intake. Although causal evidence is required to confidently support these associations, they may have future implications in primary care. Overall, the current study is one of the first to provide insight into the associations between categorical iron status groups and psychological function, encompassing both cognitive and behavioural outcomes, and the relationships that can be individually attributed to haemoglobin and serum ferritin specifically amongst women of reproductive age from a general population sample.

However, the cross-sectional nature of the study should not be overlooked as it does not allow an interpretation of cause and effect. Although controlling for potentially confounding variables reduces the amount of statistical noise, many genetic, socio-economic, and nutritional factors are shown to contribute to cognitive and behavioural function across the lifespan (Jirout et al., 2019; Kim & Park, 2017). Attributing current psychological states to current iron status is thus difficult to explicitly deduce, especially when the contribution of each variable was nominally very small. Similarly, interpreting associations between iron parameters and psychological function is challenging as it is not possible to definitively state that peripheral iron status is reflective of brain iron content. Animal studies provide evidence for nutritional ID stimulating different brain areas to acquire a distinct capacity to store iron as ferritin decreases in the hippocampus but increases in the striatum (Pino et al., 2017). However, early ID is shown to invoke long-term irreversible psychological effects; IDA in infancy significantly diminishes cognitive functioning and mood regulation in childhood (Lozoff et al., 2000; Lozoff et al., 1991), adolescence (Lozoff et al., 2000) and early adulthood (Lozoff et al., 2006; Lozoff et al., 2013) regardless of iron repletion. Neuroimaging of animal brain tissue has shown how early-life ID reduces brain iron content and alters brain tissue composition despite iron repletion (Mudd et al., 2018). This suggests that participants categorised as IS may have included those at a psychological disadvantage due to irreversible neurological damage instigated during infant ID. It may be imperative for future research to consider screening participants based on medical history to account for diagnoses of early ID. Utilising neuroimaging techniques such as magnetic resonance imaging (MRI) will also bolster interpretations surrounding iron status and psychological function to ensure that they are specific to the brain. Additionally, although cyclic variations in haematological parameters were considered for an accurate assessment of iron status at blood sampling, the study may have included participants who could have been categorised as IS one week and NAID the next. Mean serum ferritin concentrations have

been found to be highest in the luteal phase of the menstrual cycle (24.0 µg/L) compared to during menses (17.2 µg/L) (Kim et al., 1993). Using a serum ferritin cut-off of 20 µg/L as in the current study means that some participants may have fluctuated between IS and NAID classifications within one menstrual cycle and therefore between screening and testing visits. It may be imperative for future research to include an additional 'buffer' iron status group between NAID and IS groups to take into consideration those who may fluctuate between iron status classifications. Similarly, although the reproducibility of the Hemocue® is considered good, single capillary samples may be open to error whenever individual-level estimates are required (Morris et al., 1999), which may have caused some inaccuracies in individual iron status group classifications. It may therefore be necessary to use replicate sampling for measurements of haemoglobin from capillary blood in future to minimise the possibility of iron status classification errors. Additionally, it would have been beneficial to measure participants' copper status. Copper, as well as iron, is required for erythropoiesis; thus, its deficiency can cause an iron deficiency-like anaemia (Ha et al., 2016). Similarly, ceruloplasmin, a major copper carrying protein in the blood, acts as a ferroxidase to facilitate iron transport out of the cell into the circulation and to bind transferrin (Wazir & Ghobrial, 2017). Therefore, it is possible that the anaemic without iron deficiency group may have been a result of copper deficiency or that the IDA group were also deficient in copper. The interaction between copper and iron is also of interest as marginal copper deficiency is shown to greatly increase the effect of iron deficiency (Troost et al., 2003). This is especially true as all proteins involved in copper homeostasis are expressed in the brain, with key roles in antioxidant defence, energy metabolism, neurotransmitter synthesis, neuropeptide synthesis, as well as iron metabolism (Scheiber, Mercer & Dringen, 2014). Therefore, copper deficiency may also impact cognitive function, mood and fatigue in addition to the effect of iron deficiency.

Despite these limitations, the present study has established novel evidence of a greater prevalence of NAID than IDA in women of reproductive age from the general population in the United Kingdom. The null findings concerning cognitive function support earlier findings that suggest no cognitive differences across categorical iron status groups. However, in addition to heterogeneity surrounding serum ferritin cut-offs for NAID classification that make comparisons between studies challenging, previous studies have not considered upper cut-off levels for haemoglobin and serum ferritin. Consequently, iron status groupings are problematic as an IS population may include those with high haemoglobin and serum ferritin-associated cognitive deficits (Agrawal et al., 2017; Shah et al., 2009) alongside those with an 'optimal' iron status thus failing to capture meaningful and accurate relationships. The present study endeavoured to address this by implementing upper limits of haemoglobin and serum ferritin for inclusion in the per protocol population for analysis and investigating continuous

haematological markers of iron status. However, the fact that the significant positive associations of emotional functioning and MCS scores with serum ferritin did not carry over to iron status, supports the notion that the impact of serum ferritin may have been masked by categorisation, even in the absence of excess iron. It may therefore be imperative for future studies to focus upon establishing optimum haemoglobin and serum ferritin concentrations for cognitive and behavioural outcomes rather than comparing NAID and IS groups for non-pregnant women of reproductive age.

The positive associations identified between serum ferritin and mental health-related quality of life highlight the potential role of serum ferritin in emotional regulation. Moreover, the null findings concerning haemoglobin may indicate the greater significance of serum ferritin to a non-anaemic population. However, it is important to note the cross-sectional design of the study, the small contribution of the variables, and that findings are not specific to brain iron levels. Implementing a more rigorous screening of participants based upon medical history of iron status and applying neuroimaging techniques to assess brain iron levels and tissue morphology will advance this research area. Confirmation of any causative effects regarding iron status, haemoglobin and serum ferritin upon cognitive and behavioural outcomes is reliant upon well-designed iron supplementation RCTs in this population.

Chapter 3 **THE EFFECT OF IRON SUPPLEMENTATION ON COGNITION, SUBJECTIVE MOOD, FATIGUE AND WELL-BENG IN WOMEN OF REPRODUCTIVE AGE: A SYSTEMATIC REVIEW**

3.1 Introduction

Iron deficiency (ID) remains the most prevalent nutrient deficiency worldwide and is the most significant contributor to anaemia onset (World Health Organization, 2008). Growing infants and young children have the highest rates for ID and iron deficiency anaemia (IDA) (Gupta, Perrine, Mei, & Scanlon, 2017) and have consequently been the focus of most of the research in this field. However, iron insufficiency is also common in women of reproductive age due to an increased maternal and placental-foetal demand for iron during pregnancy (Brannon & Taylor, 2017; Milman, Taylor, Merkel, & Brannon, 2017) as well as increased iron depletion during regular menstruation (Harvey et al., 2005). Young females in upper-middle and high-income countries are at high risk for ID due to diets comprising an imbalance of dietary factors that inhibit iron absorption, such as consumption of phytates and polyphenols; and those that enhance it, such as ascorbic acid consumption (Beck et al., 2014). Restrictive dieting behaviours such as the avoidance of haem-rich meat also contribute to this insufficiency (Fayet, Flood, Petocz, & Samman, 2014; Young et al., 2018). Non-haematological consequences of ID include unexplained fatigue, impaired immune response, mood changes, modulation of energy metabolism and decreased cognitive performance (Beard, 2001; Murray-Kolb & Beard, 2007). Maternal ID in pregnancy can also reduce iron stores in the developing foetus causing neonatal ID (Rao & Georgieff, 2002). There is considerable evidence from animal models detailing the role of iron in neurodevelopment through its effects on cerebral energy metabolism (Dallman, 1986; Rao, Asha, Ramesh, & Rao, 2008; Ward et al., 2007), neurotransmitter synthesis (Beard & Connor, 2003; Unger et al., 2012; Youdim, Sills, Heydorn, Creed, & Jacobowitz, 1986), myelination (Badaracco, Siri, & Pasquini, 2010; Beard et al., 2003; Connor & Menzies, 1996) and neural network architecture (Brunette, Tran, Wobken, Carlson, & Georgieff, 2010; Greminger, Lee, Shrager, & Mayer-Proschel, 2014; Jorgenson, Wobken, & Georgieff, 2003). An iron insult early in life can therefore result in deficits to neurodevelopment, especially in the hippocampus, which is highly vulnerable to ID during infancy and is important for learning, memory and cognitive function (Radlowski & Johnson, 2013). This perhaps explains the relative abundance of research into the relationship between iron status and cognition in infants and children (Hermoso et al., 2011) in comparison to women of reproductive age. However, it is now recognised that the brain continues to adapt both functionally and structurally throughout the lifespan according to the changing environment (Oberman & Pascual-Leone, 2013), reflecting continued plasticity beyond early

development. These neurophysiological and neuroanatomical modifications are suggested to underpin the known shifts in cognition with age (Oberman & Pascual-Leone, 2013) leading to the possibility that changes in iron levels beyond infancy could stimulate such neural changes and subsequently modulate cognition at any time of life.

Observational studies have provided evidence for a potential benefit of iron for handling higher cognitive loads in women of reproductive age (Blanton, 2013; Blanton et al., 2013; Cook et al., 2017; Scott et al., 2017; Scott & Murray-Kolb, 2016). Longitudinal studies have also highlighted associations between self-reported ID diagnosis and decreased general health and well-being alongside increased levels of subjective fatigue (Patterson et al., 2000). However, evidence for a causal link through the conduct of intervention studies remains sparse and results are often varied. Meta-analysis of the effects of iron supplementation, regardless of iron status group, suggests significant improvements to attention, concentration and intelligence yet no significant improvements to memory, when compared to baseline (Falkingham et al., 2010). However, these findings contrast with those of a subsequent meta-analysis of the effects of iron supplementation, which is potentially due to the inclusion of both sexes aged six years and above in the former. When looking specifically at women of reproductive age, only improvements in ID participants' arithmetic scores were shown (Greig et al., 2013). A subsequent systematic review, including only ID and IDA pre-menopausal women, similarly found significant improvements in working memory as well as intellectual ability following iron supplementation. However, the effect of iron supplementation for improving mood is unclear, possibly due to a range of mood measures used for assessment leading to differing results (Lomagno et al., 2014). Greig et al. (2013) also noted that results for mental health and fatigue varied following iron supplementation, which they attributed to varying study quality.

The aim of this systematic review is to provide an updated overview of findings from randomised controlled trials investigating the effects of iron supplementation on cognition, mood, fatigue and/or well-being, specifically focussing on women of reproductive age.

3.2 Method

This systematic review was conducted in accordance with Cochrane (Higgins & Green, 2011), and was reported according to PRISMA, guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009).

3.2.1 Eligibility criteria

Eligibility criteria were determined using the PICO (population, intervention, comparison and outcome) tool. Accordingly, the population included were required to be human females between the ages of 12 and 50 years of age. Participants were also required to give baseline and endpoint blood samples measuring at least haemoglobin (Hb) and serum ferritin (sft) levels to determine iron status. Studies that met the participant criteria and included the assessment of either cognition, mood, fatigue or well-being, alongside an iron intervention versus a control arm for any duration of time were considered. Iron supplementation had to be in the form of an oral or venous supplement; high-iron diets were only considered in studies where this was used in comparison to iron supplementation. Additionally, only studies originally written in English were considered.

3.2.2 Outcome measures

In addition to the broad term of cognition, cognitive domains deemed relevant for the review included attention, executive function, memory, and reaction time (Galioto & Spitznagel, 2016). Cognitive tests must have been completed at baseline and at the end of the intervention period. Cognitive tests carried out mid-way through the intervention were also considered. Tests could be carried out by oral, written or computer test providing results were objective. Studies that assessed any domain of mood, well-being and fatigue through subjective self-report measures were considered.

3.2.3 Search strategy

To ensure all possible relevant articles were obtained for review, two researchers (HA & CHR) independently followed the same search strategy and assessed articles in accordance with the inclusion criteria. Electronic searches were initially conducted in January 2018 and updated in July 2019. The review therefore covers all studies published from the earliest record to June 30th, 2019. To guarantee adequate coverage of the literature and to avoid database bias, multiple electronic databases were searched: Cochrane Library, PubMed and Web of Science. Following the initial search, reference lists from relevant retrieved articles

were manually reviewed. The reference lists from previous systematic and meta-analytic reviews relevant to iron interventions, cognition, and subjective mood, fatigue and well-being (Falkingham et al., 2010; Greig et al., 2013; Lomagno et al., 2014) were also searched for additional studies. Following a preliminary search to determine breadth of the research topic for review, the search term *iron status* was deemed appropriate to achieve the most relevant searching algorithms for the effect of iron supplementation on cognition, mood, fatigue and well-being. Each domain was searched for separately using Boolean terms as follows: '*iron status AND…'* 1) cogniti*; 2) memory; 3) executive function; 4) reaction time; 5) attention; 6) mood; 7) well-being; 8) fatigue. The truncation symbol * used with cogniti allowed for a broader range of results, encompassing terms such as cognition, cognitive and cognitive function within one search to avoid missing relevant articles. Cogniti* and the specific cognitive domains were searched for individually to ensure accurate data capture of all relevant articles. For the mood and well-being searches, the MeSH term ("affect" [MeSH Terms] OR "affect" [All Fields]) would appear in the 'Search details' box, affecting the search output. This term was manually removed to ensure that all articles retrieved were of relevance to mood and well-being. The 'All fields' search query was utilised for all searches to provide the best opportunity to retrieve all relevant articles.

3.2.4 Study selection and data collection process

Once retrieval of all articles was complete and duplicates were removed, all remaining articles were screened independently by the researchers to determine whether they met the inclusion criteria based upon title, abstract and key words. If eligibility was unclear from the title and abstract, background and methods were examined, and a full-text screen completed if necessary. Any disagreements on study inclusion and exclusion were resolved by discussion between the reviewers. Data was extracted from baseline and endpoint for all studies. Information regarding author and published year; study design; participant characteristics (mean age, sample size at baseline); intervention details (intervention type, dosage, frequency, duration); baseline iron status parameters and criteria of iron status; number of dropouts; and outcome measures were extracted.

3.2.5 Risk of bias assessment

The use of the Cochrane risk-of-bias tool facilitated a comprehensive assessment of risk of bias in studies included in the systematic review. Risk of bias was assessed independently by the researchers (HA & CHR) addressing seven specific domains (random sequence

generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other) as either ‘high risk of bias’, ‘low risk of bias’ or ‘unclear’.

3.3 Results

3.3.1 Search results & study selection

A total of 2731 articles were retrieved through the systematic database search; of these 1077 were duplicates and thus removed. An additional five articles were retrieved through searching reference lists of previous systematic and meta-analytic reviews and articles identified through the database searches. The titles and abstracts of the remaining 1659 articles were screened for eligibility leading to the exclusion of a further 1635 articles. The main reasons for exclusion included: an inappropriate study population; animal studies; confounding disorders; no iron intervention; review paper; and no assessment of cognition/mood/fatigue/well-being. The full texts of the remaining 24 articles were read to determine inclusion; of these 11 were excluded. Three studies were excluded because high iron diets or iron bio-fortified foods were administered with no oral or venous iron supplement as a comparator (Blanton, 2013; Murray-Kolb et al., 2017; Wenger et al., 2017). Two studies were excluded as they included male and female participants but did not split results by gender (Devaki et al., 2009; Woods et al., 2014). Two studies did not include controlled interventions (Khedr et al., 2008; Kretsch et al., 1998). The remaining studies were excluded for using a demographic of pregnant women (Groner et al., 1986), an inappropriate assessment of iron status (Elwood & Hughes, 1970), including participants aged up to 55 with no specification of menstruation status (Verdon et al., 2003) and for the full text being unavailable (Swain, Penland, Johnson, & Hunt, 2006). A total of 13 articles met the inclusion criteria and were included in the review (Figure 3.1).

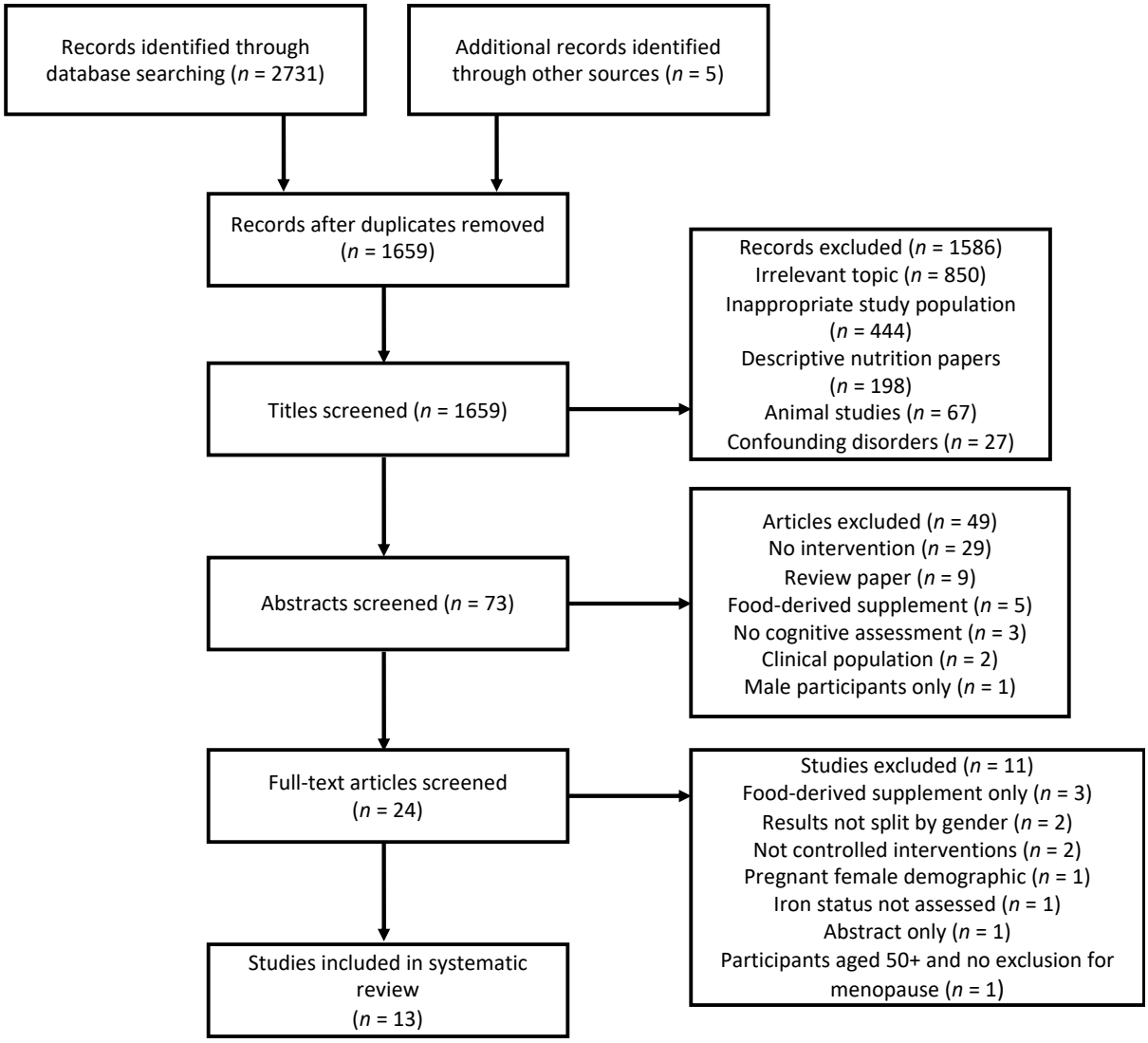


Figure 3.1 PRISMA study flow diagram for the systematic review

3.3.2 Study characteristics

Table 3.1 provides a summary of characteristics and findings of all included studies. Of the thirteen studies included all were randomised controlled trials (RCTs). Of the six studies primarily assessing cognition following iron intervention, four were conducted in high-income countries (Bruner et al., 1996; Lambert et al., 2002; Leonard et al., 2014; Murray-Kolb & Beard, 2007) and two in upper-middle income countries (Beard et al., 2005; Rezaeian, Ghayour-Mobarhan, Mazloum, Yavari, & Jafari, 2014). The seven studies primarily assessing either fatigue, mood or wellbeing were all conducted in high-income countries (Ballin et al., 1992; Favrat et al., 2014; Krayenbuehl et al., 2011; McArthur, Petocz, Caterson, & Samman, 2012; McClung et al., 2009; Patterson et al., 2001; Vaucher et al., 2012). One trial was considered to have a low risk of bias; the remainder were considered unclear overall due to unclear

processes of random sequence allocation, allocation concealment, blinding of outcome assessment, outcome data completeness and access to protocols for assessments of selective reporting (Table 3.2).

Table 3.1 Characteristics of included studies

Source	Baseline *n*, population, dropouts	Baseline iron status	Design	Dose and type of iron	Cognitive/mood tests	Results
Ballin *et al*., 1992	59 adolescent girls, 16-17 years, 0 dropouts	Any	RCT, 8 weeks	10 ml iron polystyrene sulfonate (105 mg elemental iron)	Questionnaires not specified but included questions on lassitude, fatigue, ability to concentrate, mood, appetite, quality of sleep and a physical fitness test	Iron treatment improved: subjective ability to concentrate and lassitude; mood for participants who were ID at baseline and became IS post-dose. No significant reduction in fatigue after iron supplementation.
Beard *et al*., 2005	95 mothers 6-8 weeks postpartum, 18-30 years. 14 dropouts	Iron deficiency anaemia (Hb 90-11.5 g/L, and at least 2 of the following: MCV < 80 fL; TSAT < 15 %, sft < 12 μg/L. Iron sufficient (Hb > 135 g/L; MCV > 80 fL; TSAT > 15 % and sft > 12μg/L).	RCT, 6 months	125 mg ferrous sulphate (plus 25 mg vitamin c & 10 μg folate). Controls not given iron.	Raven's Coloured Progressive Matrices, Weschler's Digit Symbol, Edinburgh Post-Natal Depression Scale (EPDS), Perceived Stress Scale, State-Trait Anxiety Inventory	Iron treatment improved: digit symbol, intelligence, depressive symptoms and perceived stress. No improvement in anxiety. Positive correlation: intelligence and Hb at baseline. Positive correlation at follow-up: intelligence, Hb, MVC and TSAT; digit symbol and MCV; stress and Hb, MCV and TSAT; anxiety, Hb and MCV. Negative correlation at follow-up: depressive symptoms, Hb and MCV.

Bruner *et al*., 1996	81 females, 13-18 years. 8 dropouts	Non-anaemic iron deficient (sft ≤12 μg/L, Hb >115 g/L (African American) >120 g/L (white))	RCT, 8 weeks	1300 mg ferrous sulphate (260 mg elemental iron) 650 mg twice daily	Brief Test of Attention; Symbol Digit Modalities Test; Visual Search and Attention Test; Hopkins Verbal Learning Test (HVLT)	Iron treatment improved: verbal working memory and verbal learning. No improvement in measures of attention. Positive correlation: change in sft and HVLT score.
Favrat *et al*., 2014	294 premenopausal women, 18+ years. 11 dropouts	Non-anaemic iron deficient (Hb ≥115 g/L, sft ≤ 50 μg/L and TSAT <20 % or sft ≤ 15 μg/L)	RCT, 1 day (followed up for 8 weeks)	One ferric carboxymaltose infusion (1000 mg iron)	Piper Fatigue Scale (PFS), SF-12, CDR system and Bond-Lader visual analogue scales	Iron treatment improved: power of attention for those with baseline sft < 15 μg/L; PFS scores at 7-days follow-up and 8-weeks; subjective mental health quality and a numerical trend for quality of physical health. No significant effects on accuracy of attention. Positive correlation: sft and improved fatigue scores. No significant associations between improved haematological measures and cognitive improvements.
Krayenbuehl *et al*., 2011	90 premenopausal women, 18+ years. 4 dropouts	Non-anaemic iron deficient (sft ≤ 50 ng/mL, Hb ≥ 120 g/L)	RCT, 2 weeks (followed up for 12 weeks)	200 mg intravenous iron (III) hydroxide sucrose	Brief Fatigue Inventory Questionnaire (BFI), Short Performance Inventory (SPI)	Iron treatment improved: SPI scores; median BFI scores decreased, and SPI scores increased for those with serum ferritin <15μg/L or transferrin saturation <20% at baseline median BFI scores significantly decreased. At follow up, significantly more of the iron treatment group reported perceived reduced fatigue than the placebo group.

Lambert *et al*., 2002	121 females, 12.5-17.9 years. 5 dropouts	Non-anaemic iron deficiency (sft <12 μg/L , Hb >120 g/l)	RCT, 8 weeks	105 mg elemental iron as tablets	Hopkins Verbal Learning Test (HVLT), Stroop Task, Visual Search, Reading Span Task	Iron treatment improved: verbal working memory and recall of learnt words from the second half of the list. No improvements in measures of attention. Positive correlation: Hb and word recall; sft and reading span.
Leonard *et al*., 2014	32 females, 18-35 years. 7 dropouts	Non-anaemic iron deficiency (sft ≤ 20 μg/L, Hb >120 g/L) Iron sufficient (serum ferritin >20 μg/L, Hb >120 g/L)	RCT, 16 weeks	60 mg or 80 mg elemental iron as ferrous sulphate	IntegNeuro battery	Iron treatment improved: impulsivity. Serum ferritin response improved: impulsivity, sustained attention and recognition memory. Haemoglobin response improved: digit-span forwards and attentional switching task scores.
McArthur *et al*., 2012	76 females, 18-35 years, 11 dropouts	Any	RCT, 12 weeks	300 mg ferrous gluconate and 200 mg ascorbic acid or pork meat diet (at least 3 pork meat meals a week)	SF-36	Iron supplementation improved: vitality Pork meat diet improved: bodily pain No significant association between measures of wellbeing and haematological measures.
McClung *et al*., 2009	219 female military soldiers, 15-25 years, 48 dropouts	Any	RCT, 8 weeks	100 mg ferrous sulphate	Two mile running time and Profile of Mood States	Iron treatment improved: vigour
Murray-Kolb and Beard, 2007	152 females, 18-35 years. 39 dropouts	Iron deficiency anaemia (Hb 105-119 g/L + ≥ 2 abnormal iron status values	RCT, 16 weeks	160 mg ferrous sulphate (60 mg elemental iron)	Cognitive Abilities Test, Shipley Institute of Living Scale	Serum ferritin response improved: attention, memory and learning cognitive domains. Haemoglobin response improved: speed of task completion.

		Non-anaemic iron deficiency (Hb ≥ 120 g/L + ≥ 2 abnormal iron status values Iron sufficient (Hb ≥120 g/L + ≤ 1 abnormal iron status value)				No significant correlation between size of sft or Hb change from baseline and cognitive improvement.
Patterson *et al*., 2001	76 females, 18-50 years, 10 dropouts	Non-anaemic iron deficient (Hb ≥90 g/L, sft <15 μg/L or sft 15-20 μg/L, plus two of the following: serum iron <10 μmol/L, TIBC >68 μmol/L or TSAT <15 %) Iron sufficient (Hb ≥120 g/L and sft >20 μg/L)	RCT, 12 weeks	350 mg ferrous sulphate or a high iron diet (2.25 mg absorbed iron per day)	SF-36 and Piper Fatigue Scale (PFS)	Iron supplementation improved: total PFS scores, behavioural/severity fatigue, affective meaning fatigue, and vitality High-iron diet improved: total PFS scores, sensory fatigue, vitality, and MCS scores No significant association between haematological measures and fatigue scores. Significantly lower vitality and mean MCS score in the treatment groups at baseline.
Rezaeian *et al*., 2014	200 females, 14-18 years. 0 dropouts	Those who had Hb ≥160 g/dL and RBC count ≥5.03 million/μl were not included	RCT, 16 weeks	50 mg ferrous sulphate twice a week	Toulouse-Piéron test	Iron treatment improved: attention span for those with IS and IDA status at baseline Positive correlation: Hb improvement and attention scores.

Vaucher *et al*., 2012	198 females, 18-50 years, 20 dropouts	Non-anaemic iron deficiency (Hb ≥ 12.0 g/dL, sft < 50 μg/L)	RCT, 12 weeks	80 mg ferrous sulphate	Current and Past Psychological Scale (CAPPS), Multidimensional Assessment of Fatigue (MAF), SF-12	Iron supplementation improved: CAPPS fatigue score, MAF global fatigue index and fatigue severity index, all independent of sft concentrations above or below 15μg/L and transferrin saturation above or below 20%. No significant improvements on measures of quality of life, depression or anxiety. Haemoglobin improvements were significantly more substantial for those with a baseline Hb <130 g/dL.

Hb, haemoglobin; IDA, iron deficient anaemic; IS, iron sufficient; MCV, mean corpuscular volume; RBC, red blood cell; RCT, randomised controlled trial; sft, serum ferritin; TIBC, total iron binding capacity; TSAT, transferrin saturation.

Source	Overall	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Ballin *et al*., 1992	?	?	?	?	?	?	?	?
Beard *et al*., 2005	?	+	?	+	?	?	?	+
Bruner *et al*., 1996	?	+	?	+	?	+	?	+
Favrat *et al*., 2014	?	+	+	+	?	?	+	+
Krayenbuehl *et al*., 2011	?	+	?	+	+	+	?	+
Lambert *et al*., 2002	?	?	?	+	?	?	?	+
Leonard *et al*., 2014	?	+	+	+	+	+	?	+
McArthur *et al*., 2012	?	+	?	+	?	?	?	+
McClung *et al*., 2009	?	?	?	+	?	?	?	+
Murray-Kolb and Beard, 2007	?	+	+	+	+	+	?	+
Patterson *et al*., 2001	?	?	?	+	?	+	?	+
Rezaeian *et al*., 2014	?	?	+	+	+	?	?	+
Vaucher *et al*., 2012	+	+	+	+	+	+	+	+

Table 3.2 Cochrane risk of bias summary

+, low risk of bias; ?, unclear risk of bias.

3.3.3 Iron status and cognitive function

Three studies found no differences in baseline cognitive function between differing iron status groups (Beard et al., 2005; Leonard et al., 2014; Rezaeian et al., 2014). One study found that iron sufficient (IS) women performed better on the cognitive tasks than those with non-anaemic ID (NAID) or IDA; however, the difference was only significant between IS and IDA groups (Murray-Kolb & Beard, 2007). Additionally, IS and NAID women completed the cognitive tasks significantly faster than those with IDA. The remaining three studies only used NAID participants, so no baseline cognitive comparisons were made (Bruner et al., 1996; Favrat et al., 2014; Lambert et al., 2002). Of the seven studies that assessed cognitive function following iron supplementation, all found significant beneficial effects.

3.3.4 Iron supplementation and cognitive function

3.3.4.1 Attention

Six studies assessed the effects of iron supplementation on attention (Bruner et al., 1996; Favrat et al., 2014; Lambert et al., 2002; Leonard et al., 2014; Murray-Kolb & Beard, 2007; Rezaeian et al., 2014) with mixed findings. Amongst females of varied iron status, post-intervention attention scores were significantly higher than baseline following iron treatment and were significantly different from the control group (Rezaeian et al., 2014). Additionally, there was a positive correlation between haemoglobin and attention scores post-iron treatment. However, amongst NAID women attention domain change scores only trended towards being significantly higher (Leonard et al., 2014) or were numerically higher (Favrat et al., 2014) for iron treatment groups than placebo controls. Two further studies did not find any significant improvements in attention following supplementation (Bruner et al., 1996; Lambert et al., 2002). However, when participants were classified as ferritin or haemoglobin responders post-intervention, significant effects were found. Ferritin responders displayed significant improvements in attention domain scores (Leonard et al., 2014; Murray-Kolb & Beard, 2007), and an improvement in power of attention (Favrat et al., 2014). Haemoglobin responders showed significantly improved speed of attention task completion (Murray-Kolb & Beard, 2007) and displayed improved accuracy on a task of attentional switching (Leonard et al., 2014).

3.3.4.2 Memory

Five studies assessed the effects of iron supplementation upon memory domains and all found significant improvements post-intervention (Beard et al., 2005; Bruner et al., 1996; Lambert et al., 2002; Leonard et al., 2014; Murray-Kolb & Beard, 2007). NAID women displayed a significant improvement in verbal working memory (Bruner et al., 1996; Lambert et al., 2002) and recognition memory change scores following iron treatment in comparison to placebo (Leonard et al., 2014). IDA mothers' digit span scores also significantly improved post-iron treatment as a reflection of short-term memory (Beard et al., 2005). Improvements in serum ferritin drove the findings of two of these studies; a significant association between change in serum ferritin and improved reading span scores was shown (Lambert et al., 2002) and better recognition memory was observed for serum ferritin responders post-intervention (Leonard et al., 2014). Ferritin responders also demonstrated seven-fold improvements in the memory domain regardless of iron status at baseline in comparison to non-responders (Murray-Kolb & Beard, 2007). Similarly, there was a significant association between greater haemoglobin and better free recall of recently heard words as a measure of verbal working memory (Lambert et al., 2002). Haemoglobin responders were also able to complete memory tasks significantly faster (Murray-Kolb & Beard, 2007) and had a significantly greater improvement on digit span forwards in comparison to non-responders (Leonard et al., 2014).

3.3.4.3 Intelligence

The only study to assess intelligence found a significant improvement in scores following a 6-month iron intervention (Beard et al., 2005). Intelligence scores of IDA mothers were almost identical to those of IS control mothers post-intervention, whilst the placebo group exhibited no change. A significant positive correlation existed between intelligence scores and haemoglobin.

3.3.4.4 Learning

The three studies that assessed the effect of iron supplementation on measures of learning found significant improvements (Bruner et al., 1996; Lambert et al., 2002; Murray-Kolb & Beard, 2007). In comparison to placebo, iron supplementation caused significant learning effects on the Hopkins Verbal Learning Test (HVLT) overall total word recall (Bruner et al., 1996) and significant improvements in words learnt from the second half of the list (Lambert et al., 2002). The former study found a significant positive correlation between change in serum ferritin and HVLT score post-intervention (Bruner et al., 1996). A five-fold improvement

in learning ability was also found for serum ferritin responders following supplementation (Murray-Kolb & Beard, 2007).

3.3.5 Iron supplementation, fatigue, mood and well-being

3.3.5.1 Fatigue

Five studies assessed perceived levels of fatigue (Ballin et al., 1992; Favrat et al., 2014; Krayenbuehl et al., 2011; Patterson et al., 2001; Vaucher et al., 2012) and four found significant improvements post-intervention (Favrat et al., 2014; Krayenbuehl et al., 2011; Patterson et al., 2001; Vaucher et al., 2012). Two studies assessed fatigue of NAID women using the Piper Fatigue Scale (PFS) (Favrat et al., 2014; Patterson et al., 2001). Total PFS scores significantly improved following oral and dietary iron interventions (Patterson et al., 2001), however the intravenous iron study extended this finding to all PFS subscales and found a significant correlation between increased serum ferritin and an improved fatigue score (Favrat et al., 2014). Another intravenous iron study of NAID women found no significant difference between treatment groups' severity of fatigue scores post-intervention but did find significant improvements in perceived fatigue change (Krayenbuehl et al., 2011). However, those with substantially depleted iron stores at baseline (serum ferritin <15 µg/L) showed significantly improved fatigue severity and fatigue change scores in comparison to placebo controls. A further study indicated that significant improvements in global fatigue and fatigue severity indices post-intervention were independent of baseline ferritin concentrations above or below 15 µg/L (Vaucher et al., 2012). Two studies followed-up their participants for eight (Favrat et al., 2014) or twelve weeks after intervention (Krayenbuehl et al., 2011) and found greater significant reductions in fatigue reported by the iron group. One study did not find any significant benefits of iron supplementation on fatigue yet found a significant improvement in lassitude; of those who reported improved lassitude, 65 % were iron deficient at baseline and were IS post-intervention (Ballin et al., 1992).

3.3.5.2 Mood and wellbeing

Quality of life, general health and well-being were assessed by two versions of the Self-Reported Health Questionnaire; SF12 (Favrat et al., 2014; Vaucher et al., 2012) and SF36 (McArthur et al., 2012; Patterson et al., 2001). Intravenous iron supplementation significantly improved NAID women's mental health quality (mental component score; MCS) whilst a numerical trend existed for subjective quality of physical health (Favrat et al., 2014). No

significant improvements in quality of life were exhibited in NAID women following oral supplementation (Vaucher et al., 2012). The SF36 was used by the two studies that included both an oral iron supplement and a high-iron diet (McArthur et al., 2012; Patterson et al., 2001). MCS numerically increased post-intervention for both treatment groups in the initial study, but this increase was only significant for the dietary intervention group (Patterson et al., 2001). The latter study found no significant MCS improvements following either intervention (McArthur et al., 2012). Vitality scores improved post-intervention in both studies; the initial study found that both NAID treatment groups showed significantly improved vitality scores from baseline to no longer be significantly lower than the IS control group at endpoint (Patterson et al., 2001) but vitality scores only significantly improved from baseline for the oral iron supplement group in the latter (McArthur et al., 2012).

Four studies broadly measured aspects of mood (Ballin et al., 1992; Beard et al., 2005; McClung et al., 2009; Vaucher et al., 2012) and all but one (Vaucher et al., 2012) found significant improvements following iron supplementation. Iron supplementation during eight weeks' basic combat training in female military soldiers significantly improved feelings of vigour from baseline in comparison to placebo (McClung et al., 2009). Another 8-week intervention found significant improvements in mood; more so for those who had been ID at baseline and became IS post-intervention (Ballin et al., 1992). However, mood was not categorised into subscales and it is not clear how and what domains of mood were subjectively assessed. Iron supplementation significantly improved depressive symptoms of IDA postpartum mothers by 25 % in comparison to the placebo group (Beard et al., 2005). A significant negative correlation was found between depression scores and haemoglobin whilst significant positive correlations were found between perceived stress and haemoglobin. Perceived stress significantly increased from baseline in the placebo and control groups, yet numerically decreased in the iron group but did not reach significance. Iron supplementation did not significantly affect anxiety levels; this is in agreement with a study on menstruating NAID women, which also found no effects on depression post-intervention (Vaucher et al., 2012).

3.4 Discussion

This systematic review aimed to synthesise and critically analyse the existing literature relating to the effect of iron supplementation upon cognition, mood, fatigue and/or well-being in women of reproductive age.

3.4.1 Cognitive function

Four studies found significant improvements in measures of attention (Favrat et al., 2014; Leonard et al., 2014; Murray-Kolb & Beard, 2007; Rezaeian et al., 2014). Two studies exhibited significant improvements in short term memory (Beard et al., 2005; Leonard et al., 2014) and one in recognition memory (Leonard et al., 2014). Furthermore, three studies demonstrated improvements in both working memory and learning ability (Bruner et al., 1996; Lambert et al., 2002; Murray-Kolb & Beard, 2007). Only one study found a significant improvement in intelligence (Beard et al., 2005). Overall, the results suggest that following iron supplementation improvements can be made upon cognitive functioning including attention, memory, and learning domains. Previous reviews have reported evidence for improvements in attention (Falkingham et al., 2010) and memory (Greig et al., 2013; Lomagno et al., 2014) following iron supplementation. Although there was evidence of improvements in intelligence from one study, this does not provide sufficient evidence in determining its impact. This is in contrast to previous systematic and meta-analytic reviews that have suggested evidence for iron supplementation improving intelligence in older children and pre-menopausal women (Falkingham et al., 2010) and pre-menopausal women alone (Lomagno et al., 2014).

Observational studies have also provided evidence to further support a role for iron in cognitive function. Correlations have been found between iron status and spatial ability (Foley, Hay, & Mitchell, 1986), attention and memory (Fordy & Benton, 1994). More recently, positive associations have been observed between body iron, serum ferritin and executive planning function in healthy, non-anaemic women of reproductive age (Blanton et al., 2013). These observational findings were replicated and extended in a subsequent dietary iron intervention study (Blanton, 2013). Despite no significant improvements in iron status parameters, higher body iron and serum ferritin were associated with significantly increased planning speed, spatial working memory and attention. These findings were corroborated by further observational results demonstrating that although iron status groups did not differ in attentional and executive function performance, higher serum ferritin and, when age was added as a covariate, higher total body iron were associated with improved planning speed (Scott & Murray-Kolb, 2016). With increasing task difficulty, change in planning speed was found to

diminish depending upon serum ferritin and total body iron concentrations; iron status could be accountable for more efficient management of difficult cognitive loads (Scott & Murray-Kolb, 2016). This review highlighted that significant ferritin and haemoglobin responses to supplementation improved attentional task performance. However, one study noted the marked increase of attention scores in comparison to haemoglobin concentrations (95 % vs 10 % increase) stimulating the suggestion that attentional improvements were a result of neurotransmitter pathway modulation rather than increased haemoglobin production and subsequent brain oxidation (Rezaeian et al., 2014). Similarly, increased iron status parameters may be indicative of oligodendrocytes obtaining a sufficient supply of iron to meet their metabolic needs for myelination thus increasing information processing speeds (Connor & Menzies, 1996). The two studies that did not find significant improvements to attention following iron supplementation suggested that the measures may not have been sensitive enough with ceiling effects evident (Bruner et al., 1996) or that findings may be a consequence of the multi-faceted nature of attention and its central role within working memory processing resources (Lambert et al., 2002). It is postulated that working memory improvements rely heavily upon attention-based processes; thus, the improvements in working memory demonstrated in these two studies may be a consequence of iron being used by attentional networks for a more efficient retrieval of working memory (Feng, Pratt, & Spence, 2012).

The current systematic literature review suggests evidence for memory, particularly working memory and short-term memory, improvements following iron supplementation in NAID and IDA women. Dopamine-dependent pathways are altered in NAID and IDA in children and young adults (Ferreira et al., 2019; Lozoff, 2011) and have been shown to return to normal following iron repletion in animal models (Nelson et al., 1997). Dopamine projections in the prefrontal cortex and hippocampus are essential for regulation of working memory through neuronal activity during task performance and for memory consolidation when learning, respectively (LaLumiere, 2014). Improvements in iron status following supplementation may facilitate dopamine regulation within these brain regions and subsequently improve memory performance. However, further investigation of this in human adults is warranted as a better iron status has previously been associated with worse performance on working memory tasks (Scott & Murray-Kolb, 2016). There is evidence to suggest that this may be by consequence of competition within an intact hippocampal system (Fretham et al., 2011), in comparison to ID-induced hippocampal memory abnormalities that may result in hippocampal avoidance and easier access and increased dominance of prefrontal systems necessary for superior task performance (Scott & Murray-Kolb, 2016).

3.4.2 Fatigue

Of the five studies that assessed fatigue following iron supplementation, four found significant positive effects post-intervention. All studies suggested a relationship between ID and increased fatigue levels. A previous meta-analytic review of NAID women included three of the studies included in this review (Favrat et al., 2014; Krayenbuehl et al., 2011; Vaucher et al., 2012); all found significant treatment effects of iron supplementation on subjective fatigue (Yokoi & Konomi, 2017). The additional study included in this review that found a significant improvement in fatigue following iron supplementation also investigated NAID women, bolstering the suggestion that iron supplementation has a serum ferritin-dependent effect upon fatigue (Patterson et al., 2001). As haemoglobin is not affected in NAID, it is postulated that serum ferritin deficits cause diminished activity of iron-dependent oxidative enzymes and respiratory proteins, reducing the extraction and utilisation of oxygen-bound haemoglobin evoking subjective and objective indications of fatigue (Bahadir et al., 2018). Similarly, fatigue onset is associated with an elevated serotonin to dopamine ratio in the brain (Davis & Bailey, 1997; Meeusen et al., 2006); monoamine dysfunction is also associated with iron deficiency presentation. The only study not to find a significant improvement in fatigue had no eligibility criteria regarding iron status resulting in unequal numbers of iron status within and between treatment groups (Ballin et al., 1992), which may have contributed to the lack of significant findings. A causal link between IDA and increased subjective fatigue is well established, whilst this link amongst NAID women is becoming increasingly acknowledged (Dziembowska et al., 2019; Houston et al., 2018).

These findings are in accordance with meta-analytic results suggesting a benefit of iron supplementation for improving physical exercise performance in women of reproductive age (Pasricha et al., 2014) and NAID female athletes (Burden et al., 2015). A recent meta-analysis however found that physical performance only improved when a serum ferritin cut-off of ≤ 20 µg/L and below was used (Rubeor et al., 2018), contrary to the studies assessing subjective fatigue in this review. This suggests that subjective fatigue is responsive to iron supplementation at higher serum ferritin concentrations, whilst physical performance may only be amenable at lower levels.

3.4.3 Mood and wellbeing

The effect of iron supplementation on improving aspects of mood and wellbeing appears to be far more complex. Of the seven studies assessing mood and wellbeing, six found significant effects; however, these are somewhat divergent. Quality of life, as assessed by the SF-12,

improved following an intravenous iron supplement (Favrat et al., 2014) but not following oral iron supplementation (Vaucher et al., 2012). A single total dose of intravenous iron is shown to be as beneficial as a daily oral iron supplementation (Iyoke et al., 2017), and the same dose of intravenous iron given daily is more effective than daily oral iron (Bhavi & Jaju, 2017). This would give support to a beneficial effect of intravenous iron over daily oral iron for improved perceived health and well-being, however further investigation is warranted. Mental health, as assessed by the SF-36, significantly improved following a dietary intervention of lean beef or lamb (Patterson et al., 2001) but not following a dietary pork intervention (McArthur et al., 2012). Although all are classed as red meat, unprocessed beef and lamb have a higher mean iron content than pork with only beef qualifying as a source of iron in accordance with European regulations (Cashman & Hayes, 2017). Dietary pork was able to maintain haemoglobin concentrations to the same extent as supplemental iron; however, serum ferritin only increased following supplemental iron (McArthur et al., 2012). This may explain why improvements in vitality were only significant when either a beef-rich diet or an oral iron supplement was consumed, coupled with significant improvements in haemoglobin and serum ferritin.

A significant improvement in depressive symptoms was only found amongst IDA postpartum mothers (Beard et al., 2005). Low ferritin and/or IDA is identified as a risk factor for postpartum depression (PPD) occurrence (Wassef et al., 2019). Postpartum iron supplementation significantly improves PPD symptoms over six weeks (Sheikh et al., 2017) and is shown to reduce PPD risk, however no protective effects are observed when iron is administered during pregnancy (Wassef et al., 2019). Monoaminergic systems involved in mood regulation are dysregulated during iron deficiency (Kim & Wessling-Resnick, 2014) and amongst postpartum women (Xie et al., 2018; Yildiz et al., 2017); the combined effect may subsequently exacerbate depressive symptoms. However, no significant improvements in depressive or anxiety symptoms were shown in NAID women in this review (McClung et al., 2009; Vaucher et al., 2012). A relationship between depression and low serum ferritin concentrations, in non-anaemic women has been observed previously. In comparison to healthy females, serum ferritin was significantly lower in individuals with depression; this was reflected by a significantly higher frequency of ID (<15 µg/L) in females with depression (Vahdat Shariatpanaahi et al., 2007). Depressive symptoms may not have improved in these intervention studies due to a higher serum ferritin at baseline; the first study had no inclusion criteria for serum ferritin (McClung et al., 2009) whilst the latter used a higher cut-off of < 50 µg/L resulting in only 32.4 % of the iron treatment group having serum ferritin < 15 µg/L (Vaucher et al., 2012). Further investigation is warranted focussing upon NAID women, to

determine whether depressive symptoms can successfully be ameliorated in this demographic.

3.4.4 Evaluation

This systematic review has expanded upon previous reviews regarding the impact of iron supplementation upon cognition in women of reproductive age. Yet it is the first to focus on RCTs of mood, fatigue and well-being regardless of whether cognition was also being measured. Subsequently, this allowed the inclusion of an additional five studies and a more thorough discussion of the literature was possible. Similarly, the review has expanded knowledge in the field for women of reproductive age specifically, avoiding including studies in infants and children (Falkingham et al., 2010), and has focussed on iron supplementation alone rather than additional nutritional supplementation types (Lomagno et al., 2014). The search was not limited by date as all studies published and recorded from Cochrane Library, Web of Science and PubMed were included from the earliest date to the current year.

However, there are several factors that must be taken into consideration when basing conclusions on the results of this review. Firstly, heterogeneity surrounding the methods chosen to assess and categorise participants into iron status groups was evident. To not be considered anaemic, it is recommended that haemoglobin concentrations are ≥120 g/L (World Health Organization, 2011b); the studies included in this review that consider NAID to include those with a haemoglobin concentration >90 g/L are possibly open to misinterpretation (Favrat et al., 2014; Patterson et al., 2001). Similarly, serum ferritin cut-offs for ID vary between organisations and laboratories worldwide (Daru et al., 2017). In this review, serum ferritin ranged from <12 to ≤50 µg/L for NAID classification. Identifying a cut-off for serum ferritin reflective of depleted iron stores remains challenging due to its acute-phase protein (APP) properties; consequently, there are no well-defined diagnostic criteria (Namaste et al., 2017; Soppi, 2018). Subsequently, serum ferritin concentrations can be elevated following an inflammatory response to microbial invasion, tissue injury, chronic or autoimmune diseases (Northrop-Clewes, 2008), which can lead to an inaccurate calculation of iron stores. Obesity and exercise-associated inflammation are also shown to stimulate reactive oxygen species production, which prompts increases in hepcidin production to cause degradation of the transmembrane protein ferroportin instigating reduced iron absorption (Aigner et al., 2014; Domínguez et al., 2018; Gaffney-Stomberg & McClung, 2012). It is recommended that other APPs, such as C-reaction protein and alpha-1-glycoprotein, are controlled for to determine whether an acute phase response is present and reduce diagnostic inaccuracies (Suchdev et al., 2017). However, seven of the 13 studies included studies did not control for the potential

effect of inflammation (Ballin et al., 1992; Bruner et al., 1996; Lambert et al., 2002; McArthur et al., 2012; McClung et al., 2009; Patterson et al., 2001; Rezaeian et al., 2014). Concurrently, although the majority of the included studies controlled for chronic and autoimmune diseases, the same cannot be said for body mass index (BMI) or physical activity.

There was also a lack of consistency regarding supplementation duration, method of administration and dosage. The intervention period ranged from two weeks to 6 months despite the recommended supplementation duration being 12 to 24 weeks to restore and improve iron status (Baird-Gunning & Bromley, 2016). Consequently, half of the studies included in this review would not fulfil this criterion. Similarly, administration of the supplement varied; eleven studies administered iron orally (9 ferrous sulphate; 1 ferrous gluconate; 1 iron polystyrene sulphonate) whilst two used intravenous iron. Despite oral iron, specifically ferrous sulphate, being the gold-standard treatment for ID (Santiago, 2012), its daily administration to a non-clinical population is associated with an increased risk of gastrointestinal side effects when compared to placebo or intravenous iron delivery (Tolkien et al., 2015). This encourages reductions in treatment compliance, though associated adverse effects and compliance were not consistently documented in the included studies of this review increasing the difficulty of determining the tolerability of the interventions.

Iron supplement dosage also varied across the studies, from 37.4 mg-260 mg elemental iron. A comparative study included in this review identified an elemental 60 mg iron dose to be as effective as an 80 mg dose for improving iron status in NAID women with no significant difference in adverse events or compliance (Leonard et al., 2014). However, 60 mg is, substantially greater than the recommended daily allowance of 14.8-16.0 mg/day for non-pregnant, menstruating women (Milman, 2019), and greater than the tolerable upper level of iron intake of 40-45 mg for gastrointestinal discomfort prevention (Pra et al., 2012). Resolution of NAID has been achieved after an 8-week intervention of 26 mg daily iron supplementation without the occurrence of adverse effects (D'Adamo et al., 2018). A lower dose supplement administered over a longer duration may be more beneficial for achieving an optimal iron status and reducing adverse effects.

Dietary iron intake at baseline was considered by less than half of the studies included in this review. Although possible to achieve optimal iron intake without consuming meat, both haem iron and non-haem iron are positively associated with serum ferritin concentrations in healthy young women, with haem iron exhibiting a stronger association (Young et al., 2018). Red meat-rich diets may therefore be of increased value to maintaining optimal iron status. These findings are echoed by positive associations between consumption of animal flesh foods and

iron status (Jackson et al., 2016) and meta-analytic findings suggesting vegetarians have significantly lower serum ferritin compared to non-vegetarians and are subsequently more susceptible to ID (Haider et al., 2018). Higher serum ferritin concentrations are also associated with tertiary education (Young et al., 2018), which may be a consequence of increased health awareness and desire for greater diet quality, especially when following a diet low in haem iron. A higher quality diet is associated with a greater executive functioning (Cohen, Gorski, Gruber, Kurdziel, & Rimm, 2016) and a reduced prevalence of depressive and anxiety symptoms in women (Gibson-Smith, Bot, Brouwer, Visser, & Penninx, 2018; Quehl, Haines, Lewis, & Buchholz, 2017; Sakai, Murakami, Kobayashi, Suga, & Sasaki, 2017), bolstering the need to assess not only dietary iron intake but also overall diet quality.

Similarly, there was a lack of control for the menstrual cycle prior to haematological testing, group allocation and randomisation. Women of reproductive age are at a greater risk of ID due to increased iron losses as a result of menstrual bleeding (Johnson et al., 2016). Quantitative measures of menstrual blood loss have proven to significantly predict iron status (Harvey et al., 2005), whilst subjective approaches have identified significantly lower serum ferritin concentrations for those with greater menstrual blood loss (Heath et al., 2001; Toxqui et al., 2014). It is therefore important to take menstrual blood loss into consideration when assessing iron status and methods of combating ID. Similarly, no studies controlled for where participants were in their menstrual cycle prior to collecting haematological samples. Haemoglobin and serum ferritin are at their lowest during menses and at their highest during the luteal phase of the menstrual cycle (Kim et al., 1993). It is thus recommended that iron biomarkers should be assessed during the late luteal phase of the menstrual cycle for accurate iron status interpretation (Laine et al., 2016).

3.4.5 Conclusion

Overall, there is evidence to suggest that iron supplementation can improve cognitive function, particularly attention, memory and learning, in addition to subjective fatigue in menstruating women of reproductive age. However, the impact of iron on subjective mood and well-being appears much more complex, which may be due to the heterogeneity surrounding intervention routes of administration, intervention doses and the methods chosen to assess and categorise participants into iron status groups across the included studies. Further research including assessments of response to iron supplementation and controlling for confounding variables of dietary iron intake, BMI, physical activity, menstrual cycle and blood loss are needed to confirm and extend these results.

Chapter 4 **EFFICACY EVALUATION OF 16 WEEKS' DIETARY SUPPLEMENTATION WITH IRON BIS-GLYCINATE ON COGNITIVE FUNCTION, MOOD, FATIGUE AND WELLBEING**

4.1 Introduction

The systematic review reported in Chapter 3 indicated significant benefits of iron supplementation for improving iron status, cognitive function, mood, fatigue and well-being in non-pregnant women of reproductive age. However, not all iron RCTs have considered baseline iron status (Ballin et al., 1992; McArthur et al., 2012; McClung et al., 2009). Concerning NAID, significant improvements to iron status, measures of memory (Bruner et al., 1996; Lambert et al., 2002; Leonard et al., 2014; Murray-Kolb & Beard, 2007), attention (Favrat et al., 2014; Leonard et al., 2014; Murray-Kolb & Beard, 2007), learning (Bruner et al., 1996; Murray-Kolb & Beard, 2007), fatigue (Favrat et al., 2014; Krayenbuehl et al., 2011; Patterson et al., 2001; Vaucher et al., 2012) and well-being (Favrat et al., 2014; Patterson et al., 2001) following iron supplementation in NAID women of reproductive age have been observed, inferring a causal relationship between iron and psychological functioning. However, it is noteworthy that the cut-offs for NAID varied across the included studies; serum ferritin ranged from < 12 to < 50 µg/L whilst haemoglobin ranged from > 90 to ≥ 120 g/L. Worldwide clinical guidelines propose a haemoglobin concentration of ≥ 120 g/L to signify non-anaemia in women (World Health Organization, 2011a), which suggests the studies classifying NAID using haemoglobin < 120 g/L (Favrat et al., 2014; Patterson et al., 2001) may be misrepresenting IDA as NAID. Consequently, studies only including NAID women may be limited to a small proportion of the population with low serum ferritin or could have included participants with serum ferritin concentrations greater than those considered reflective of ID. Similarly, IS groups could encompass participants with performance deficits associated with high haemoglobin or serum ferritin, which could lead to inaccuracies in comparisons of iron supplementation between NAID and IS women. To address this issue, it may be important to recruit women of reproductive age into iron supplementation RCTs based on NAID and IS iron status to ensure a range of iron status across non-anaemic women in the absence of excess iron, while controlling for baseline serum ferritin concentration. As haemoglobin is positively associated with folate due to its involvement in the formation of iron-containing haem (Elema, Yimam, Waka, & Olana, 2018; Koury & Ponka, 2004), serum ferritin (a measure of iron stores) is required to determine iron status, especially amongst a non-anaemic population. Controlling for baseline serum ferritin, alongside CRP to account for its APP properties, would allow for the impact of baseline iron status on any treatment effects to be accounted for whilst overcoming the problems associated with iron status group categorisations.

The RCTs that have used a cut off of ≤ 20 μg/L for NAID in studies recruiting both NAID and IS women (Leonard et al., 2014; Murray-Kolb & Beard, 2007) both administered oral ferrous sulphate, the gold-standard iron preparation for IDA treatment (Cook, 2005; Santiago, 2012; Tolkien et al., 2015), for 16 weeks, which is within the recommended 12 to 24 weeks required to significantly replenish iron stores in the brain (Baird-Gunning & Bromley, 2016). However, both administered elemental iron doses (60-80 mg) much greater than the recommended daily allowance of 14.8-16.0 mg for non-pregnant, menstruating women (Milman, 2019), and greater than the tolerable upper level of iron intake of 40-45 mg based upon the prevention of gastrointestinal discomfort (Pra et al., 2012). Additionally, iron doses exceeding 30 mg are shown to increase the likelihood of gastrointestinal discomfort (Low et al., 2016), whilst meta-analysis suggests that ferrous sulphate causes significant gastrointestinal side-effects amongst adults (Tolkien et al., 2015) such as nausea, vomiting, abdominal discomfort, constipation and dark-coloured stools (Johnson-Wimbley & Graham, 2011). Consequently, adherence to treatment protocols is often affected as once haemoglobin concentrations are improved or corrected after four weeks, iron supplementation must continue for approximately three months to replenish serum ferritin (Alleyne, Horne, & Miller, 2008; Goddard et al., 2011). Of the two comparable RCTs from the systematic review, one reported analogous side effects and compliance between treatment and placebo groups (Leonard et al., 2014), however such findings were not reported in the other (Murray-Kolb & Beard, 2007). Following this, investigations of alternate sources of iron at doses below 30 mg have been trialled; 26 mg of elemental iron cultured from yeast led to significant increases in serum ferritin concentrations in NAID women of reproductive age without adverse effect presentation (D'Adamo et al., 2018). It is thus imperative to investigate lower and more tolerable doses of iron to determine whether they too can improve psychological functioning yet in a more efficient manner than higher doses of elemental iron from ferrous formulations.

Another option to reduce adverse effects of ferrous formulations is to use a different iron preparation. As described in section 1.6.3, iron bis-glycinate has a heterocyclic ring structure, which infers a stronger stability constant than that of inhibitory ligands and prevents the ferrous cation from interacting with the gastric mucosal lining. This leads to augmented iron bioavailability for absorption and improved gastrointestinal tolerability in comparison to ferrous formulations (Ashmead, 2001). Consequently, IS non-pregnant women of reproductive age show a significantly greater preference for iron bis-glycinate over ferrous sulphate following trends for lower incidence of gastrointestinal adverse effects (Coplin et al., 1991) and significantly fewer reports of adverse effects (Fouad et al., 2013). This promising evidence has prompted its use as an alternative oral supplement for IDA treatment. Research has targeted

populations of infants, young children and adolescents due to the increased demand for iron and increased risk of IDA during these stages of life. A dose-response study that focused on IDA treatment in adolescents discovered that 30 mg iron bis-glycinate chelate was as effective as 120 mg ferrous sulphate in improving iron status (Oscar Pineda et al., 1994). Increased bioavailability of the chelate was associated with greater efficacy for increasing serum ferritin concentrations at 60 mg and 120 mg in comparison to 120 mg ferrous sulphate. Similarly, fewer gastric complaints were reported in all iron bis-glycinate groups with no complaints reported by the 30 mg group. For IDA treatment in infants, equal doses of iron bis-glycinate and ferrous sulphate (5 mg/kg/day) significantly increased haemoglobin after 28 days, however iron bis-glycinate resulted in a significantly greater increase in serum ferritin (Pineda & Ashmead, 2001); the bioavailability of iron bis-glycinate was 3.4 times higher than ferrous sulphate. Lower doses of iron bis-glycinate chelate (0.75 mg/kg/day) have also demonstrated an efficacy comparable to a four-time greater dose of ferrous sulphate in new-born children (Bagna et al., 2018) for IDA prevention. Additionally, the efficacy of iron bis-glycinate for IDA treatment in children has been compared to polymaltose iron (Name et al., 2018), which has similarly enhanced bioavailability and tolerability compared to ferrous formulations (Jacobs, Wood, & Bird, 2000; Jacobs, Wormald, & Gregory, 1979; Ortiz et al., 2011; Saha, Pandhi, Gopalan, Malhotra, & Saha, 2007; Toblli & Brignoli, 2007; Yasa et al., 2011). Following a 45-day intervention, both treatments (3 mg/kg/day) led to significantly improved haemoglobin concentrations. However, only iron bis-glycinate prompted significantly increased serum ferritin concentrations, which highlights the greater efficacy of the compound for replenishing iron stores at low doses and over a short intervention duration (Name et al., 2018). Such findings demonstrate the superiority of iron bis-glycinate chelate over ferrous sulphate formulations and polymaltose iron for IDA resolution with improved tolerability.

Due to the marked increase in serum ferritin associated with iron bis-glycinate treatment, investigations of its efficacy have extended to children who are NAID to further knowledge on the prevention of IDA. An inverse relationship between serum ferritin and the absorption of iron bis-glycinate chelate has been observed (Bovell-Benjamin et al., 2000; Olivares et al., 1997). A 12-week intervention with 30 mg/day iron bis-glycinate chelate or ferrous sulphate in children with normal-range haemoglobin and serum ferritin <12 ug/L found a significant improvement in serum ferritin for both treatment groups (Duque et al., 2014). Iron bis-glycinate was more efficient than ferrous sulphate for sustaining serum ferritin 6 months after supplementation. It is postulated that the persistence of this effect is because of the ability to overcome the negative impact of iron inhibitory compounds present in the diet (Duque et al., 2014).

One other approach to overcoming absorption issues associated with ferrous salts has been to co-supplement with ascorbic acid (Vitamin C), which is shown to enhance the absorptive properties of ferrous formulations and accelerate improvements to iron status (Chiamchanya, 2013). Ferrous formulations combined with ascorbic acid have been compared to iron bis-glycinate chelates for IDA management during pregnancy. A 100 mg dose of iron bis-glycinate significantly improved haemoglobin from baseline after 14 and 28 days; this improvement was also significantly greater at day 28 compared with that of ferrous ascorbate treatment (Kamdi & Palkar, 2015). For serum ferritin, significant improvements were evident after 14 and 28 days in both treatment groups but improvements were significantly greater at both time points for iron bis-glycinate. This demonstrates the higher bioavailability and efficacy of the bis-glycinate chelate over a ferrous formulation for the management of IDA in pregnant women, even when the ferrous salt is combined with an enhancer of iron absorption like ascorbic acid. Despite the greater dose in comparison to previous studies, 28 days of treatment did not restore haemoglobin concentrations to non-anaemic classification warranting extended intervention durations. To date, however, no iron supplementation studies have investigated the co-supplementation of iron bis-glycinate and ascorbic acid. Considering the beneficial effect ascorbic acid has on iron bis-glycinate absorption in an iron inhibitor-rich environment (Olivares et al., 1997), it is imperative to investigate whether such co-supplementation would improve the already superior bioavailability of iron bis-glycinate supplements to subsequently improve iron status further.

Largely, although NAID is more prevalent amongst women of reproductive age than IDA, comparatively less is known about the effects of iron supplementation in NAID women. Previous iron RCTs in NAID women of reproductive age have adopted varying cut-off levels for serum ferritin, resulting in studies that have either only focussed on a small proportion of the population who have very low serum ferritin or have considered NAID at cut-offs greater than levels considered reflective of ID. To address this, investigations of a range of NAID and IS women of reproductive age are warranted that take baseline serum ferritin into account when assessing treatment effects. Additionally, it appears that the designs of the RCTs conducted so far could be improved by supplementing with a low-dose iron bis-glycinate chelate following observed improvements to bioavailability, efficacy, tolerability and subsequent compliance compared to ferrous formulations. Despite the advantages of iron bis-glycinate, no studies to date have investigated its administration for improving iron status in non-pregnant women of reproductive age with NAID. The inverse relationship between iron bis-glycinate chelate absorption and serum ferritin suggests the potential of iron bis-glycinate to be of most benefit to a NAID population. To date, iron bis-glycinate chelate has not been investigated to assess cognitive and mood parameters. It would be valuable to determine

whether the improvements to psychological and physiological function highlighted in the preceding chapter's systematic review, are replicated or extended because of the increased bioavailability and tolerability of low doses of iron bis-glycinate chelate. Additionally, the increased absorption of iron by co-supplementation of iron bis-glycinate chelate and ascorbic acid warrants investigation to determine whether it will be more advantageous to psychological and physiological health than iron bis-glycinate alone. The present study therefore aims to build upon the methodologies of previous RCTs to compare the effects of low-dose iron bis-glycinate and iron bis-glycinate plus ascorbic acid treatments on cognitive function, mood, fatigue and well-being. The present study will investigate the effects of 16 weeks supplementation with either 28 mg/d of iron bis-glycinate chelate alone, 28 mg/d iron bis-glycinate chelate plus 240 mg ascorbic acid or matched placebo in women of reproductive age upon the same cognitive, mood, fatigue and wellbeing measures assessed in Chapter 2.

4.2 Methods

4.2.1 Design & ethics

This study employed a randomised, placebo-controlled, double-blind, stratified groups design, with participants randomly assigned to one of three treatment groups (placebo, iron, iron and vitamin C; see section 4.2.3). The study received ethical approval from Northumbria University's Psychology Department and was conducted according to the Declaration of Helsinki (1964). The study was registered on www.clinicaltrials.gov under the identifier NCT04469010.

4.2.2 Participants

One hundred and fifty-one females aged 18-49 years were recruited into the study following completion of the study that formed the basis of Chapter 2 and who met the inclusion criteria described in section 2.2.2[9]. In addition, participants reported themselves to not have any food allergies or sensitivities relevant to the interventional product and had a haemoglobin concentration ≥120 g/L. An a priori power analysis was conducted to calculate the sample size based upon a mixed measures ANCOVA-F test with medium effect size (f = 0.25) using G Power 3.1.9.2. This indicated that a total sample of 141 participants with 47 participants per treatment arm was required to detect a significant difference at an alpha level of 0.05 between groups to achieve power of .75. All participants were non-anaemic and were randomised into each treatment arm by stratified sampling to ensure a range of NAID and IS participants across treatments. To account for potential dropouts, the total sample size was increased to a total sample of 151 participants to include at least 150 participants.

4.2.3 Treatments

All participants were randomly assigned to either placebo, 28 mg/day iron bis-glycinate chelate, or 28 mg/day iron bis-glycinate chelate plus 240 mg vitamin C within their stratified groups of IS or NAID. The manufacturer (Bayer HealthCare – Consumer Care, Basel, Switzerland) prepared all treatments in identical bottles; these were coded as A, B or C and

[9] A visual representation of the participant disposition throughout the studies that comprise this thesis can be found in Appendix V.

were delivered to the lab in boxes organised by treatment code. The lead researcher had no access to identification materials associating these letters to the respective treatment. A stratified randomisation schedule was created by the second supervisor using randomization.com. Treatment bottles within each box were relabelled with a treatment randomisation number that corresponded to their iron status stratification group (IS 101-178; NAID 201-273) according to the randomisation schedule, assigning each participant to an A, B or C treatment. Upon completion, the principal supervisor ensured that the treatment bottles in the A, B and C boxes corresponded with the randomisation schedule. Treatment bottles were assigned to participants in sequential order stratified by iron status group. Treatments were oblong tablets identical in appearance to ensure participants and the research team remained blind to which treatment had been dispensed. Upon completion of all analyses in the thesis, the lead researcher and principal supervisor were unblinded to allow data interpretation with the knowledge of which treatment corresponded to the A, B and C codes.

Participants were randomly allocated to a single treatment for the duration of the study according to a randomisation schedule. Treatments were provided in two bottles of 63 tablets to be consumed once per day for 16 weeks. The treatment duration mimicked the protocols of previous iron intervention studies (Leonard et al., 2014; Murray-Kolb & Beard, 2007) following evidence of a reduced rate of iron recovery in the brain in comparison to the liver following treatment in animal models (Youdim et al., 1989). To combat the known increased risk of gastrointestinal side effects associated with traditional ferrous formulations of iron supplements that reduce tolerability and successful iron absorption, the active treatments comprised iron bis-glycinate chelate as the source of iron. Participants were instructed to commence taking their treatment the morning following randomisation with a glass of water and to avoid co-ingesting with milk, coffee, tea or orange juice due to the known respective reducing and enhancing absorptive properties. This was especially important due to one of the active treatments co-supplementing iron with vitamin C. Participants were advised to take their treatment immediately upon wakening and to avoid food or drink for one hour after. The final tablet was ingested the morning before participant's final testing visit (Week 16) to conclude the supplementation period.

4.2.4 Demographic/lifestyle questionnaires

The current study utilised the same demographic/lifestyle questionnaires of caffeine consumption, physical activity, subjective nutrient and food group frequencies and menstrual blood loss outlined in section 2.2.3.

4.2.5 Cognitive and behavioural function assessment battery

The current study utilised the same cognitive and behavioural function assessment battery outlined in sections 2.2.4 and 2.2.6.

4.2.6 Treatment guess questionnaire and compliance

Upon completion of the study, participants were provided with a treatment guess questionnaire (APPENDIX VI). This required participants to choose between whether they believed they had been randomised to an active or placebo treatment for the duration of the study and to provide a reason for this choice. A chi-square test was used to analyse the correct and incorrect responses given by treatment group for confirmation of the blinding procedure.

A treatment compliance percentage was calculated upon study completion to ensure appropriate treatment consumption by participants in accordance with the study protocol. This percentage was calculated by counting the number of returned tablets and comparing it to the number of tablets that should have been consumed based on the number of days between the first and final study visits for each participant.

4.2.7 Blood sampling

In addition to the samples outlined in section 2.2.7, additional venous samples were collected for vitamin C and zinc. During the screening visit for the study described in Chapter 2, baseline venous samples were obtained, with post-dose samples obtained during the Week 16 assessment. Any samples obtained from participants who were not enrolled in the current study were destroyed.

4.2.7.1 Vitamin C

Biological analysis of plasma vitamin C is considered the most reliable measure of vitamin C status (Dehghan, Akhtar-Danesh, McMillan, & Thabane, 2007; Travica et al., 2017). International population studies have established vitamin C status limits; deficiency (<11 µmol/L), depletion or marginally deficient (11 - 28 µmol/L), adequate (28 - 40 µmol/L) and optimal (>40 µmol/L) to the upper cut-off point of 170 µmol/L (Hampl, Taylor, & Johnston, 2004).

Blood samples were obtained via venepuncture into lithium heparin vacutainers (4 ml). Samples were inverted 8 - 10 times, refrigerated at 5 °C, and processed within 2 hours of acquisition. The plasma was separated in a refrigerated centrifuge (4 °C, 10 minutes, 1200 rpm/277 x g) after which, 0.5 ml of plasma was transferred into two 1.5 ml Eppendorf tubes® in addition to an equal volume of 10 % metaphosphoric acid for stability as a protein-free acid supernatant (Lykkesfeldt, 2002). The sample was centrifuged again under the same conditions and immediately frozen at -80 ^{0}C prior to high performance liquid chromatography (HPLC) analysis conducted on-site.

Following the procedure of Robitaille and Hoffer (2016), samples were thawed over ice and 50 µL of plasma was placed in 1 ml plastic Eppendorf tubes®. To prepare the samples for analysis, an equal volume of 5 mmol/L tris(2-carboxy ethyl) phosphine hydrochloride (TCEP) in water was added and the sample allowed to react for 20 minutes at room temperature before centrifuging (4 °C, 5 minutes, 16000 x g). The supernatant was kept on ice and transferred into autosampler vials and injected immediately onto the HPLC column. The sample injection volume was 20 µL and samples were maintained at a temperature of 4 °C.

The analysis was carried out using an Agilent 1260 Infinity modular HPLC system equipped with a multi-wavelength detector set to 245 nm. The column was a Thermo Hypersil BDS C18 (150 x 4.6 mm, 5 µm) maintained at a temperature of 25 °C. The mobile phase was 1.8 mM sulphuric acid (aq.) and the flow rate was 1 mL/min. Total run time was 10 minutes and the retention time of the vitamin C peak was 2.5 minutes. Each sample run through the HPLC system included a plasma quality control sample to ensure internal batch precision following the standard curve developed using a peak areas linear regression from six ascorbic acid standards ranging from 0 to 100 µmol/L. The assay is a highly sensitive and reproducible HPLC method for the determination of ascorbic acid concentrations in human plasma (Robitaille & Hoffer, 2016).

4.2.7.2 Zinc

Zinc was measured to monitor levels across the intervention period in response to iron supplementation as zinc shares some of its absorptive and transport mechanisms with iron, which may promote competition between the minerals (Sandstrom, 2001). Biological analysis of plasma or serum zinc concentrations is the most widely used measure of zinc status (Wieringa, Dijkhuizen, Fiorentino, Laillou, & Berger, 2015). For women of reproductive age (18 - 49 years) cut offs for zinc deficiency are dependent on fasting and time of day; <10.7 µmol/L

(morning, fasting), <10.1 μmol/L (morning, non-fasting) and 9 μmol/L (afternoon, non-fasting) (Grønli, Kvamme, Friborg, & Wynn, 2013; Wieringa et al., 2015).

Blood samples were obtained via venepuncture into Trace Element Serum (TE) vacutainers (6 ml). Samples were inverted 8-10 times, refrigerated at 5 °C, and allowed to clot for at least 30 minutes before they could be processed within 4 hours of acquisition. Samples were centrifuged at 3000 rpm (1734 x g) for 10 minutes at 4 °C. Following centrifugation, 1 ml of plasma was poured off into two 1.5 ml Eppendorf tubes® and immediately frozen at -80 ^{0}C prior to analysis conducted by Newcastle Laboratories, Newcastle-upon-Tyne.

Samples were analysed by inductively coupled plasma-mass spectroscopy (ICP-MS) in collision mode. ICP-MS is a multi-element analytical technique capable of trace level elemental analysis. For zinc (m/z 66) isotope measurements, serum samples are diluted with Butan-1-ol and diluent containing yttrium for internal standardisation. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6,000-8,000 °C. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure) from the mass spectrometer (vacuum). The ions pass through a focusing region and then the universal reaction cell. Analysis of zinc is in Kinetic Energy Discrimination Mode (KED) with helium as the collision gas. The ions pass through the quadrupole mass filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of zinc to be determined. The quadrupole is sequentially scanned to specific mass to charge ratio of each analyte and intensity is detected with a pulse detector. Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element.

4.2.8 Procedure

The procedure outlined in section 2.2.8 was followed for all participants enrolled. Prior to their testing assessment for the study described in Chapter 2, participants eligible for the current study were informed of their eligibility and sent all study information. Following completion of the procedure outlined in section 2.2.8, the requirements of the study were discussed in line with the participant information sheet supplied to the participant prior to screening. Following

informed consent, participants received the first bottle of their allocated treatment along with instructions on how to take them and to commence supplementation the following morning. Participants were provided with a treatment diary (APPENDIX VII) to log the time and date of ingested tablets, to note any missed tablets, any concomitant medications and any adverse events they may have experienced during the supplementation period.

Participants returned to the lab during week 8 to collect the final bottle of their allocated treatment. During this visit, participants returned their diary and any remaining tablets so that treatment compliance could be calculated, and any concomitant medications and adverse events noted in the Case Report Form (CRF). A new diary was provided to participants to complete in an identical manner for weeks 8 - 16 of the supplementation period.

The week 16 assessment followed the same protocol as the baseline assessment with the exception of the return of their diary and all remaining tablets, the completion of the demographic/lifestyle questionnaires (excluding the food frequency questionnaire) initially completed at screening for the study described in Chapter 2, and a treatment guess questionnaire. Participants were asked if they had significantly changed their dietary habits over the supplementation period (e.g., adopted a vegan diet), which was noted down as a procedural deviation if so. Venous and finger-prick blood samples were obtained at week 16 to determine a profile of iron, vitamin C, zinc and oestrogen (not analysed due to COVID-19) status. Upon completion of the final testing assessment, participants were fully debriefed.

4.2.9 Data cleaning

One hundred and fifty-one participants were enrolled into the study of which 135 completed all requirements. Of the 16 participants who did not complete the study; seven were lost to follow up; three withdrew due to time commitments; two withdrew due to self-reported lack of compliance; two withdrew as they believed they were taking placebo; one withdrew due to gastrointestinal upset and one withdrew due to falling pregnant. Of those who completed the study, 70 were IS and 65 NAID at baseline. The per-protocol analysis excluded six participants for a treatment compliance below 80 %; five for elevated CRP (>10 mg/L); three for donating blood the week prior to their final testing visit; one for elevated serum ferritin in the absence of inflammation (>150 µg/L confers a risk of iron overload in the general population (World Health Organization, 2011b)) and one for haemoglobin above the normal range of 120-150 g/L (Lewis et al., 2004).

Prior to conducting the analyses, the data cleaning procedures detailed in section 2.2.9 were followed for the removal of anomalous and outlier data. Data were cleaned by stratification group so that any expected differences between iron status and treatment groups were accounted for. Additionally, normality of data distribution was checked by plotting residual change scores and producing histograms for inspection. Residual change scores that deviated from the normal distribution were removed. Upon completion of these procedures for each outcome variable assessed, data analysis commenced.

4.2.10 Statistical methods

Baseline differences across treatment groups were investigated using one-way ANOVAs. Analysis across baseline and post-dose was then conducted in two phases via linear mixed models using the MIXED procedure in SPSS. Linear mixed models were used instead of ANCOVAs as they are more advantageous for longitudinal datasets, including the ability to account for missing data points often encountered and the ability to model non-linear, individual characteristics (Krueger & Tian, 2004). The main effect of treatment group (placebo, iron, iron and vitamin C) was analysed. Treatment was included as a fixed factor in the model and respective baseline values were entered as a covariate. Additionally, baseline serum ferritin was included as a covariate to account for the impact of baseline iron status on any treatment effects. All post-hoc analyses compare both active treatments to placebo only and are Sidak corrected comparisons.

4.3 Results

4.3.1 Participants

The population for analysis consisted of 63 IS and 56 NAID participants. Participant disposition through the trial can be found in Figure 4.1 and their demographic data by treatment group in Table 4.1.

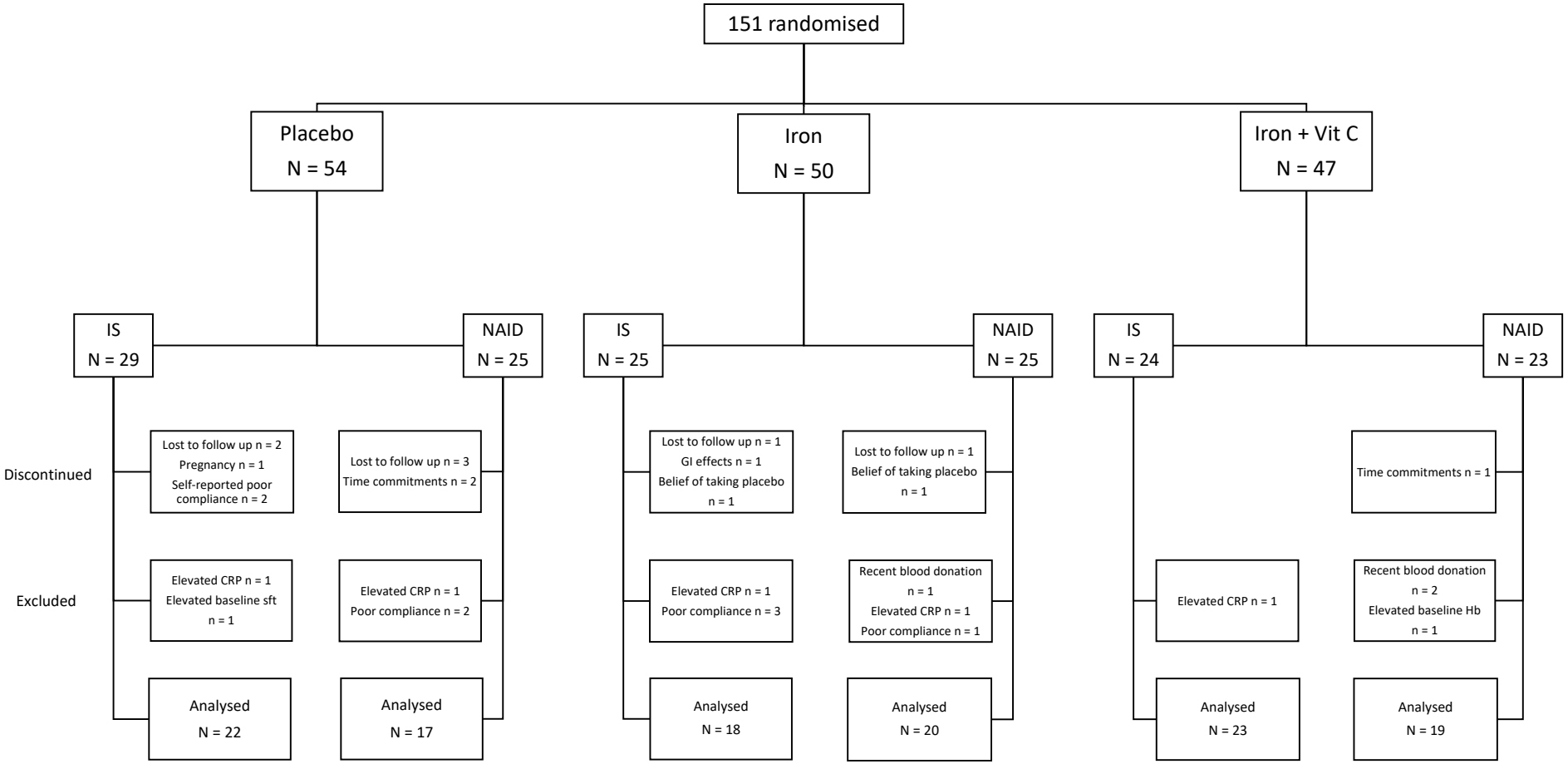


Figure 4.1 Participant disposition through the trial. Figure depicts the final disposition of participants throughout the study, culminating in N = 119 of the 151 randomised. IS = iron sufficient; NAID = non-anaemic iron deficient; GI = gastrointestinal; CRP = C-reactive protein; Hb = haemoglobin; sft = serum ferritin

Table 4.1 Participant demographic information and baseline characteristics. Means and Std. Deviation (SD) are presented with F and p values of the main effects from the one-way ANOVAs conducted on the baseline data by treatment group

		N	Baseline		Main effects	
			Mean	SD	*F*	*p*
Haemoglobin (g/L)	Placebo	39	126.92	6.51	1.80	.170
	Iron	38	127.74	6.39		
	Iron + Vit C	42	125.10	6.35		
Serum ferritin (µg/L)	Placebo	39	37.90	30.83	.594	.554
	Iron	38	34.61	28.81		
	Iron + Vit C	42	31.26	22.19		
Vitamin C (µmol/L)	Placebo	25	15.95	5.71	.257	.774
	Iron	26	17.02	7.39		
	Iron + Vit C	37	16.96	5.24		
Zinc (µmol/L)	Placebo	26	10.72	1.45	.293	.747
	Iron	23	10.60	1.67		
	Iron + Vit C	30	10.93	1.70		
Age (years)	Placebo	39	27.79	8.86	.543	.582
	Iron	38	27.74	8.82		
	Iron + Vit C	42	26.10	7.41		
Years in education	Placebo	39	17.74	3.45	2.23	.112
	Iron	38	16.47	2.18		
	Iron + Vit C	42	16.79	2.51		
BMI (kg/m^2)	Placebo	39	23.75	3.36	.762	.469
	Iron	38	24.62	3.94		
	Iron + Vit C	42	24.57	3.21		
Systolic BP (mmHg)	Placebo	39	114.82	10.83	1.08	.344
	Iron	38	118.38	11.73		
	Iron + Vit C	42	116.48	9.40		
Diastolic BP (mmHg)	Placebo	39	76.67	7.25	1.15	.319
	Iron	38	78.89	7.19		
	Iron + Vit C	42	78.77	7.45		
Menstrual blood loss score[10]	Placebo	**30**	**2.58**	1.47	**4.40**	**.015**
	Iron	**30**	**4.30***	2.88		
	Iron + Vit C	33	3.56	2.23		
Physical activity (MET minutes)	Placebo	38	3025.28	1504.66	1.96	.145
	Iron	42	4076.80	2687.74		

[10] Menstrual blood loss was only calculated for those with a menstrual cycle; hence the reduced N. Higher scores are indicative of increased menstrual blood loss.

	Iron + Vit C	38	3540.81	2543.17		
Cereals and cereal products (g/day)	Placebo	39	245.32	99.61	.667	.515
	Iron	38	244.71	139.79		
	Iron + Vit C	42	220.91	81.67		
Dietary iron (mg/day)	Placebo	39	10.95	3.65	.044	.957
	Iron	38	10.81	4.20		
	Iron + Vit C	42	10.71	3.30		
Fish and fish products (g/day)	Placebo	39	43.13	44.18	.869	.422
	Iron	38	37.28	38.03		
	Iron + Vit C	42	32.08	30.23		
Meat and meat products (g/day)	Placebo	39	95.26	58.23	.016	.984
	Iron	38	96.23	70.68		
	Iron + Vit C	42	93.58	70.33		
Nuts and seeds (g/day)	Placebo	39	12.39	20.67	.028	.972
	Iron	38	11.18	16.19		
	Iron + Vit C	42	11.77	28.06		
Vegetables (g/day)	Placebo	39	306.82	180.05	.261	.771
	Iron	38	335.04	169.29		
	Iron + Vit C	42	319.43	165.30		
Alcohol consumption (units/day)	Placebo	39	1.04	1.21	.316	.730
	Iron	38	0.86	0.84		
	Iron + Vit C	42	0.93	0.97		
Caffeine consumption (mg/day)	Placebo	38	192.74	163.14	.617	.541
	Iron	38	219.12	182.28		
	Iron + Vit C	42	180.29	128.42		

* = significant difference between the placebo and active treatment group below $p < .05$

4.3.2 Treatment effects

4.3.2.1 Demographic/lifestyle variables

A significant effect of treatment for menstrual blood loss was identified [F (2, 68) = 3.39, p = .039]. Post hoc comparisons revealed significantly greater menstrual blood loss scores for the placebo group (3.30) than the iron and vitamin C group (2.43; p = .030), however no significant difference was identified between the iron (3.05; p = .740) and placebo groups (Table 4.2). However, this should be interpreted with caution due to not all participants having a menstrual cycle and changes in menstruation status from baseline to post-dose.

The analysis identified no significant effects of treatment for BMI or physical activity.

Table 4.2 Demographic/lifestyle variable outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose			Main Effects	
		n	Mean	SE	Mean	SE	df	F	p
	Placebo	25	2.58	0.27	**3.30**	**0.24**			
Menstrual blood loss	Iron	24	4.04	0.47	3.05	0.25	**2, 68**	**3.39**	**.039**
	Iron + Vit C	24	3.56	0.39	**2.43***	**0.24**			
	Placebo	32	23.75	0.54	24.54	0.13			
BMI	Iron	34	24.62	0.64	24.50	0.12	2, 99	.319	.728
	Iron + Vit C	37	24.57	0.49	24.41	0.12			
	Placebo	36	3025.28	312.90	3019.67	332.92			
Physical activity (IPAQ)	Iron	37	3824.47	317.10	3267.99	328.78	2, 107	.147	.864
	Iron + Vit C	39	3292.34	301.23	3192.19	319.56			

* = significant difference between placebo and active treatment group below p < .05.

4.3.2.2 Cognitive domain analysis

The analysis identified no significant effects of treatment for cognitive domain performance. See Table 4.3 for baseline and post-dose means and standard error (See Appendix VIII for outputs).

Table 4.3 Cognitive task analysis outcomes[11] for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Episodic Memory Accuracy	Placebo	39	0.03	0.12	-0.04	0.09	2, 114	.782	.460
	Iron	38	-0.17	0.10	-0.09	0.09			
	Iron + Vit C	42	0.06	0.11	0.07	0.09			
Episodic Memory Speed	Placebo	38	0.14	0.15	-0.05	0.10	2, 110	.683	.507
	Iron	36	0.03	0.16	0.04	0.10			
	Iron + Vit C	41	-0.03	0.14	-0.12	0.09			
Executive Function Accuracy	Placebo	39	-0.04	0.11	0.06	0.11	2, 114	.352	.704
	Iron	38	0.09	0.10	0.03	0.11			
	Iron + Vit C	41	-0.05	0.10	-0.06	0.10			
Executive Function Speed	Placebo	39	-0.03	0.11	0.06	0.09	2, 114	.735	.482
	Iron	37	0.13	0.11	0.06	0.09			
	Iron + Vit C	41	-0.05	0.13	-0.07	0.09			
Working Memory Accuracy	Placebo	39	0.02	0.13	-0.01	0.10	2, 113	.424	.655
	Iron	38	-0.19	0.14	0.02	0.10			
	Iron + Vit C	42	0.13	0.10	-0.11	0.10			
Working Memory Speed	Placebo	37	0.16	0.16	0.01	0.11	2, 99	.079	.924
	Iron	37	0.01	0.16	-0.04	0.12			
	Iron + Vit C	41	-0.14	0.16	-0.05	0.11			
Sustained Attention Accuracy	Placebo	39	0.04	0.13	-0.19	0.12	2, 113	1.58	.210
	Iron	37	-0.03	0.15	0.00	0.12			
	Iron + Vit C	42	-0.07	0.12	0.09	0.11			
Sustained Attention Speed	Placebo	39	-0.04	0.14	0.12	0.08	2, 113	2.12	.125
	Iron	37	0.11	0.15	-0.11	0.09			
	Iron + Vit C	42	-0.00	0.13	0.09	0.08			
Learning	Placebo	38	0.12[a]	0.12	0.10	0.11	2, 105	2.49	.087
	Iron	35	-0.27[ab]	0.14	-0.18	0.11			
	Iron + Vit C	37	0.14[b]	0.08	0.15	0.11			

[a] = significant difference between the placebo and an active treatment group at baseline below $p < .05$; [b] = significant difference between the active treatment groups at baseline below $p < .05$

[11] For accuracy outcomes, positive values are indicative of better performance and negative values of worse performance. For speed outcomes, positive values are indicative of slower speed and positive values of slower speed.

4.3.2.3 Subjective Mood Analysis

4.3.2.3.1 POMS

A significant effect of treatment for depression-dejection scores was identified [F (2, 96) = 5.27, p = .007]. Post hoc comparisons revealed significantly higher ratings of depression-dejection in the placebo group (2.37) compared to the iron group (0.79; p = .004) and a trend towards significantly higher ratings compared to the iron and vitamin C group (1.38; p = .071) (Figure 4.2). See Table 4.4 for baseline and post-dose means and standard error.

Additionally, a significant effect of treatment for total mood disturbance was identified [F (2, 89) = 3.21, p = .045]. Post hoc comparisons revealed significantly higher ratings of total mood disturbance in the placebo group (12.44) compared to the iron group (4.39; p = .048) and a trend towards significantly higher ratings compared to the iron and vitamin C group (5.36; p = .079) (Figure 4.2).

See Table 4.4 for baseline and post-dose means and standard error.

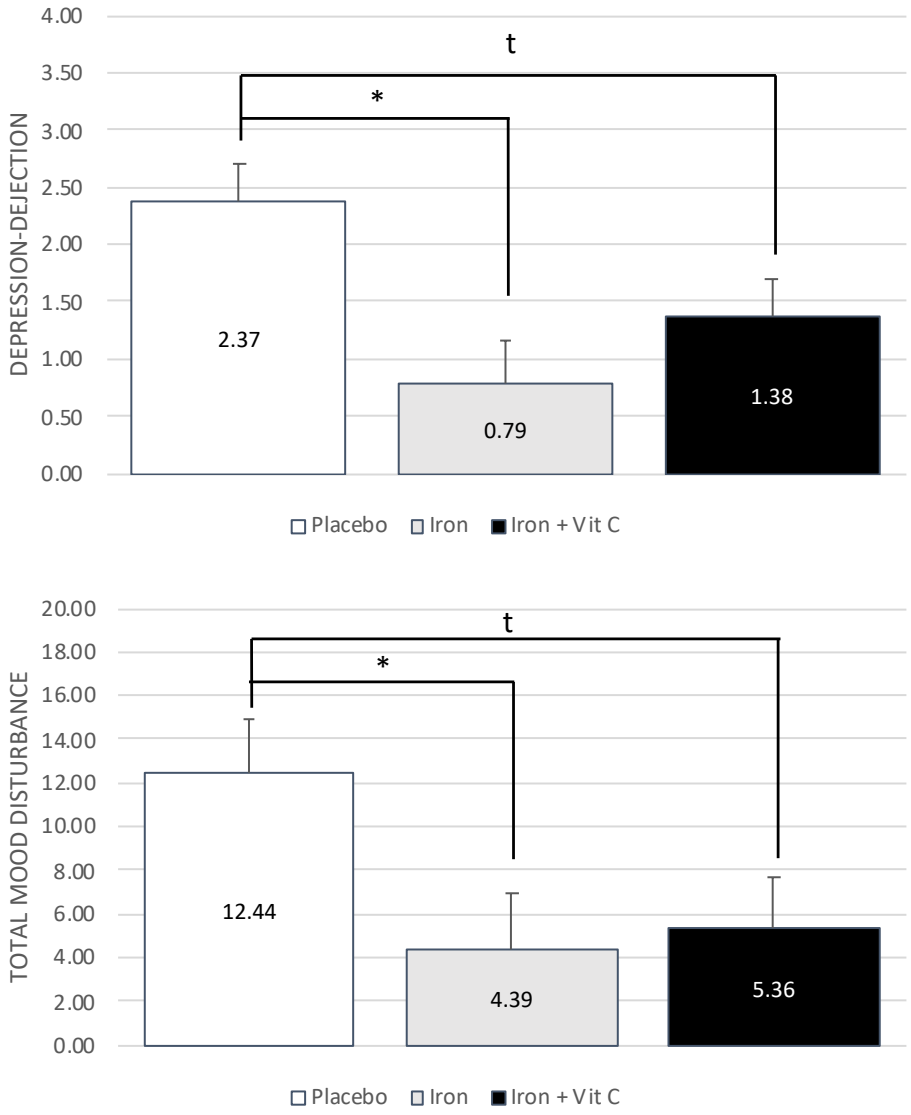


Figure 4.2 Estimated marginal means and standard error (SE) for post-dose values of depression-dejection **(top)** and total mood disturbance **(bottom)** by treatment group (*= $p < .05$; t= $p < .10$)

Table 4.4 Subjective mood analysis (POMS) outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
	Placebo	34	5.44	0.57	5.13	0.60			
Tension-Anxiety	Iron	32	5.00	0.55	5.01	0.62	2, 99	.619	.541
	Iron + Vit C	38	5.05	0.43	4.28	0.57			
	Placebo	34	1.81	0.35	**2.37**	**0.34**			
Depression-Dejection	**Iron**	29	2.42	0.55	**0.79***	**0.37**	**2, 96**	**5.27**	**.007**
	Iron + Vit C	38	1.10	0.38	1.38^{T}	0.32			
	Placebo	33	1.86	0.39	1.56	0.31			
Anger-Hostility	Iron	33	1.66	0.32	0.96	0.31	2, 97	1.38	.257
	Iron + Vit C	36	1.25	0.29	0.91	0.30			
	Placebo	36	12.39	0.96	12.46	0.73			
Vigour-Activity	Iron	37	13.03	1.05	13.84	0.72	2, 107	.947	.391
	Iron + Vit C	39	12.49	0.96	12.96	0.70			
	Placebo	37	6.84	0.79	6.98	0.57			
Fatigue-Inertia	Iron	35	7.63	1.05	5.65	0.59	2, 107	1.79	.172
	Iron + Vit C	40	6.17	0.64	5.67	0.55			
	Placebo	35	8.57	0.73	8.41	0.53			
Confusion-Bewilderment	Iron	35	9.28	1.01	7.53	0.53	2, 103	1.44	.241
	Iron + Vit C	38	7.49	0.58	7.19	0.51			
	Placebo	31	10.47	2.52	**12.44**	**2.44**			
Total Mood Disturbance	**Iron**	29	7.77	3.61	**4.39***	**2.52**	**2, 89**	**3.21**	**.045**
	Iron + Vit C	34	6.92	2.10	5.36^{T}	2.34			

* = significant difference between placebo and active treatment group below $p < .05$; ^{T} = trend towards a significant difference between placebo and active treatment group below $p < .10$

4.3.2.3.2 PSS and SCI

The analysis identified no significant effects of treatment for perceived stress or subjective sleep quality. See Table 4.5 for baseline and post-dose means and standard error.

Table 4.5 Subjective mood analysis (PSS and SCI) for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Perceived Stress	Placebo	39	19.74	1.00	19.09	0.78	2, 110	.318	.728
	Iron	36	22.11	1.13	19.79	0.81			
	Iron + Vit C	40	19.12	1.25	19.90	0.76			
Sleep Quality	Placebo	39	19.46	1.14	20.05	0.87	2, 108	.400	.672
	Iron	36	17.94	1.41	18.94	0.89			
	Iron + Vit C	38	17.22	1.08	19.58	0.88			

4.3.2.3.3 NASA-TLX

The analysis identified no significant effects of treatment for subjective workload. See Table 4.6 for baseline and post-dose means and standard error.

Table 4.6 Subjective workload analysis (NASA-TLX) outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Total Workload	Placebo	38	52.83	2.13	52.23	1.81	2, 111	.332	.718
	Iron	38	56.52	1.83	50.53	1.80			
	Iron + Vit C	42	51.94	1.85	50.40	1.71			

4.3.2.4 Subjective fatigue (PFS and VAS)

The analysis identified no significant effects of treatment for subjective fatigue. See Table 4.7 for baseline and post-dose means and standard error.

Table 4.7 Subjective fatigue analysis (PFS and VAS) outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Behavioural-Severity	Placebo	37	3.13	0.31	2.79	0.23	2, 108	.179	.172
	Iron	37	3.59	0.34	2.19	0.23			
	Iron + Vit C	39	3.08	0.29	2.59	0.22			
Affective Meaning	Placebo	39	4.69	0.33	4.31	0.34	2, 113	2.32	.103
	Iron	38	4.94	0.36	3.61	0.34			
	Iron + Vit C	41	4.87	0.31	4.61	0.33			
Sensory	Placebo	38	5.48	0.33	5.08	0.30	2, 113	2.72	.070
	Iron	38	5.48	0.33	4.09	0.30			
	Iron + Vit C	42	5.70	0.26	4.65	0.29			
Cognition-Mood	Placebo	38	4.66	0.30	4.19	0.23	2, 111	1.79	.171
	Iron	37	4.51	0.28	3.59	0.23			
	Iron + Vit C	41	4.54	0.23	3.98	0.22			
Total Fatigue	Placebo	38	4.43	0.29	3.97	0.24	2, 113	2.38	.097
	Iron	38	4.61	0.29	3.33	0.24			
	Iron + Vit C	42	4.48	0.23	3.97	0.23			
Mental Fatigue	Placebo	35	58.14	2.69	58.65	2.95	2, 107	.502	.607
	Iron	36	59.18	2.87	56.21	2.89			
	Iron + Vit C	41	62.10	2.29	54.62	2.73			
Alertness	Placebo	35	42.08	2.84	41.82	3.08	2, 109	1.73	.183
	Iron	38	38.39	2.70	48.67	2.95			
	Iron + Vit C	41	42.00	2.33	42.05	2.84			

4.3.2.5 Subjective wellbeing (SF-12)

A significant effect of treatment for physical component scores was identified [*F* (2, 112) = 5.78, *p* = .004]. Post hoc comparisons revealed significantly lower scores in the iron group (56.27) compared to the placebo group (58.52; *p* = .012), however no significant difference for the iron and vitamin C group (58.67; *p* = .977) compared to placebo (Figure 4.3). See Table 4.8 for baseline and post-dose means and standard error.

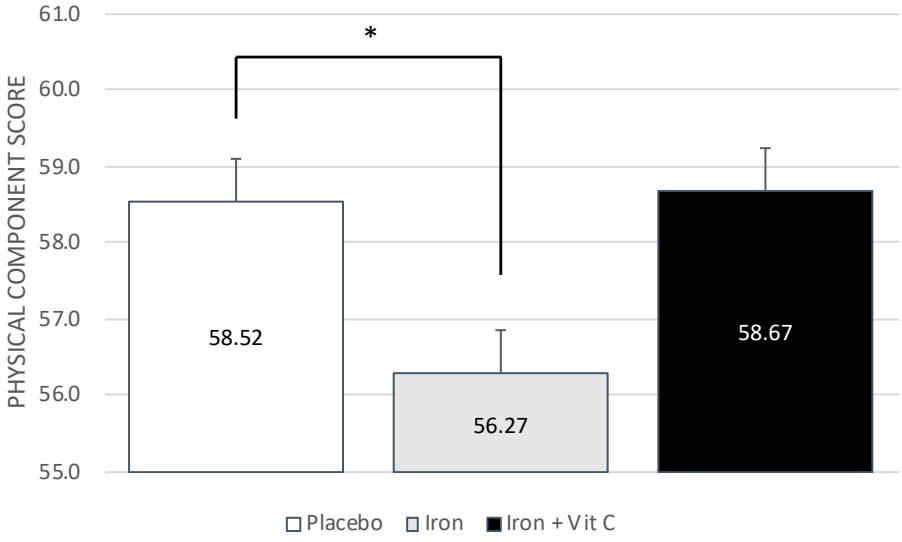


Figure 4.3 Estimated marginal means and standard error (SE) for post-dose values of physical component scores by treatment group (*= p <.05)

Table 4.8 Subjective wellbeing (SF12) analysis outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Physical Functioning	Placebo	39	55.24	0.84	55.80	0.41	2, 111	1.20	.306
	Iron	36	54.78	0.66	55.74	0.43			
	Iron + Vit C	41	54.44	1.06	56.54	0.40			
Role-Physical	Placebo	39	53.01	0.97	54.53	0.60	2, 111	.596	.553
	Iron	37	51.67	1.21	54.36	0.62			
	Iron + Vit C	40	54.24	0.79	55.25	0.60			
Bodily Pain	Placebo	39	53.34	1.14	55.06	0.71	2, 112	2.93	.058
	Iron	37	54.17	0.99	53.14	0.72			
	Iron + Vit C	41	54.94	0.78	55.39	0.69			
General Health	Placebo	38	53.56	1.27	54.63	0.97	2, 113	1.03	.362
	Iron	38	52.15	1.43	54.80	0.98			
	Iron + Vit C	42	54.80	0.81	53.05	0.93			
Vitality	Placebo	38	49.59	1.33	50.88	1.04	2, 113	1.63	.201
	Iron	38	48.81	1.32	52.52	1.04			
	Iron + Vit C	42	51.41	1.25	49.93	1.00			
Social Functioning	Placebo	38	49.18	1.35	50.28	1.05	2, 113	.905	.407
	Iron	38	47.54	1.34	51.66	1.05			
	Iron + Vit C	42	51.40	1.17	52.18	1.01			
Role- emotional	Placebo	39	46.29[a]	1.18	46.57	1.35	2, 114	.726	.486
	Iron	38	40.69[ab]	1.91	48.46	1.40			
	Iron + Vit C	42	47.12[b]	1.63	46.26	1.31			
Mental health	Placebo	38	46.09	1.14	45.03	1.24	2, 113	1.24	.293
	Iron	38	42.77	1.31	47.82	1.25			
	Iron + Vit C	42	46.18	1.23	46.41	1.18			
PCS	**Placebo**	38	56.80	0.84	**58.52**	**0.57**	**2, 112**	**5.78**	**0.04**
	Iron	38	57.84	0.87	**56.27***	**0.57**			
	Iron + Vit C	41	57.56	0.77	58.67	0.55			
MCS	Placebo	38	44.18	1.22	43.92	1.27	2, 113	2.35	.100
	Iron	38	39.76[b]	1.58	47.64	1.29			
	Iron + Vit C	42	45.50[b]	1.54	44.57	1.22			

* = significant difference between placebo group and an active treatment group below p < .05; [a] = significant difference between the placebo and an active treatment group at baseline below $p < .05$; [b] = significant difference between the active treatment groups at baseline below $p < .05$

4.3.2.6 Biochemical analysis

A significant effect of treatment for haemoglobin was identified [F (2, 114) = 3.95, p = .022]. Post hoc analyses revealed significantly higher levels of haemoglobin in the iron and vitamin C group (133.07; p = .023) and a trend towards significantly higher levels in the iron group (132.45; p = .050) compared to the placebo group (127.15) (Figure 4.4).

A significant effect of treatment for serum ferritin was also identified [F (2, 109) = 9.19, p < .001]. Post hoc analyses revealed significantly greater serum ferritin concentrations for both the iron group (54.69; p = .004) and the iron and vitamin C group (59.00; p < .001) compared to the placebo group (38.80) (Figure 4.4).

Additionally, a significant effect of treatment for vitamin C was identified [F (2, 52) = 6.67, p = .003]. However, post-hoc comparisons revealed no significant differences between the iron group (9.32; p = .075) or the iron and vitamin C group (17.35; p = .354) compared to the placebo group (14.56).

The analysis identified no significant effects of treatment for zinc levels.

See Table 4.9 for baseline and post-dose means and standard error.

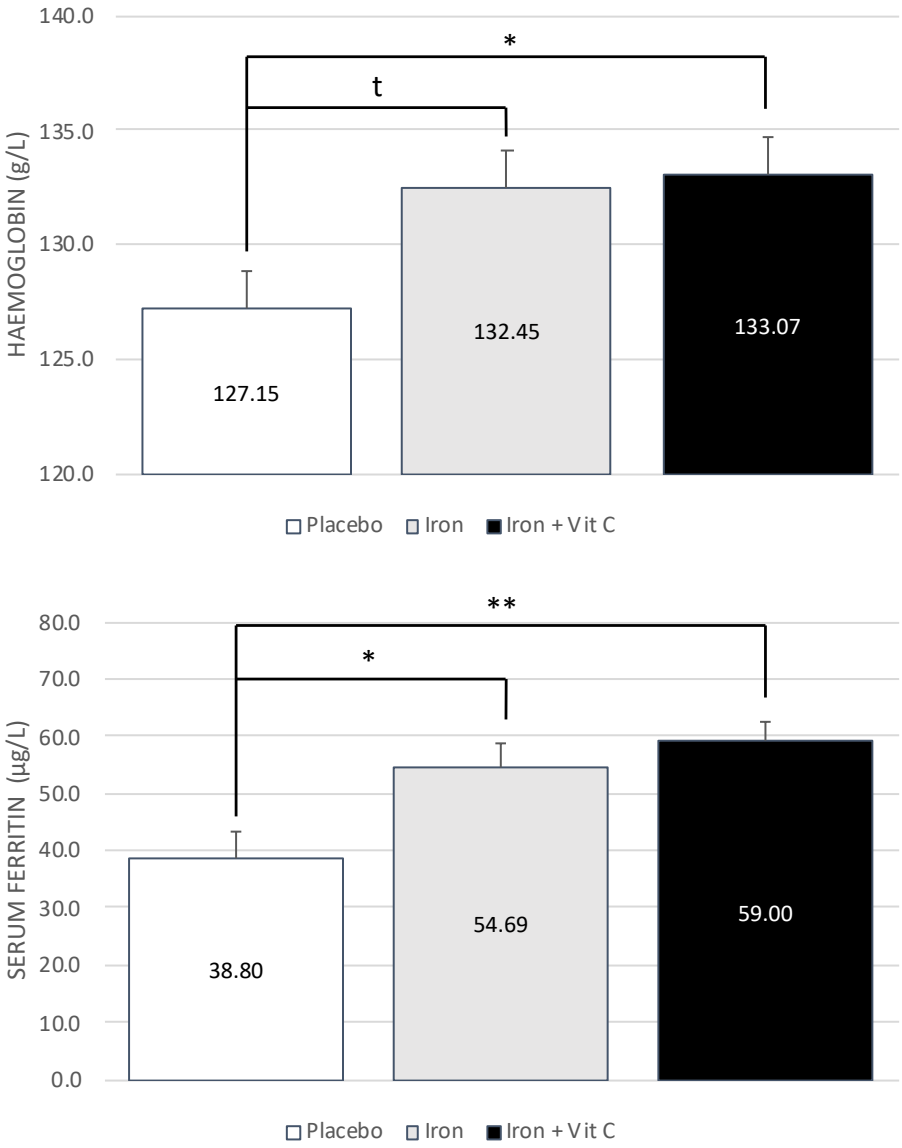


Figure 4.4 Estimated marginal means and standard error (SE) for post-dose values of haemoglobin **(top)** and serum ferritin **(bottom)** by treatment group (*= $p < .05$; **= $p < .001$; t= $p < .10$)

Table 4.9 Biochemical analysis outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects		
		n	Mean	SE	Mean	SE	df	F	p
Haemoglobin (g/L)	**Placebo**	39	126.92	1.04	**127.15**	**1.64**	**2, 114**	**3.95**	**.022**
	Iron	38	127.74	1.04	132.45^{T}	1.67			
	Iron + Vit C	42	125.10	0.98	**133.07***	**1.60**			
Serum ferritin (µg/L)	**Placebo**	37	37.90	4.94	**38.80**	**4.39**	**2, 109**	**9.19**	**< .001**
	Iron	37	34.61	4.67	**54.69***	**4.25**			
	Iron + Vit C	39	31.26	3.42	**59.00****	**3.62**			
Vitamin C (µmol/L)	Placebo	17	15.95	1.14	14.56	1.73	**2, 52**	**6.66**	**.003**
	Iron	15	17.02	1.45	9.32^{T}	1.87			
	Iron + Vit C	25	16.96	0.86	17.35	1.39			
Zinc (µmol/L)	Placebo	15	10.72	0.29	12.69	0.42	2, 34	.715	.496
	Iron	9	10.60	0.35	12.21	0.54			
	Iron + Vit C	15	10.93	0.31	13.03	0.42			

* significant difference between placebo group and an active treatment group below $p < .05$; ** significant difference between placebo group and an active treatment group below $p < .001$; ^{T} trend towards a significant difference between placebo group and an active treatment group below $p < .10$.

4.3.3 Compliance and adverse events

Two participants did not return their unused treatments. For the remaining participants, compliance was observed to be excellent in all three groups (101 % Placebo; 100 % Iron; 100 % Iron & Vit C) with a one-way ANOVA identifying no significant difference for compliance percentage by treatment group [F (2, 116) = .274, p = .761]. Sixty-four percent of participants in the placebo group believed that had received placebo compared to 45 % and 44 % of participants in the iron and iron and vitamin C groups, respectively. Chi-squared analysis confirmed this to be a non-significant difference [χ^2 (2) = .794, p = .672].

A chi-square test was conducted on the adverse events reported in response to treatment guess and revealed no significant association between treatment and adverse event reporting [χ^2 (2) = 3.35, p = .187]. See Table 4.10.

Table 4.10 Frequency of adverse events reported via treatment diary over the 16-week intervention period by treatment group

Treatment	Adverse event							
	Headache	Stomach/bowel problems	Sore throat	Fever	Muscle pain	Migraine	Nosebleed	Total
Placebo	32	5	2	1	0	2	0	42
Iron	26	4	2	0	0	0	1	33
Iron & Vit C	22	2	0	0	3	0	0	27

4.4 Discussion

Overall, it was found that both iron alone and iron and vitamin C supplementation were effective for improving iron status parameters. Compared to placebo, iron and vitamin C significantly improved haemoglobin and serum ferritin concentrations whilst iron alone only significantly improved serum ferritin concentrations. Iron alone significantly reduced PCS compared to placebo, indicating lower physical health. Nevertheless, supplementation with iron alone significantly reduced ratings of depression-dejection and total mood disturbance compared to placebo. Furthermore, the iron and vitamin C treatment significantly reduced menstrual blood loss scores compared to placebo. No significant effects of treatment compared to placebo were identified for cognitive domain performance, subjective workload, perceived stress, sleep quality, subjective fatigue, and vitamin C or zinc levels.

The current study did however find that iron treatment alone significantly reduced ratings of depression and total mood disturbance, whilst this effect trended numerically for iron and vitamin C supplementation. This is suggestive of a causal role of iron in mood regulation in non-anaemic women of reproductive age. These findings expand on those from previous RCTs that did not find significant improvements in depressive mood following higher iron doses in NAID women of reproductive age who presented with considerable fatigue (Vaucher et al., 2012; Verdon et al., 2003). This is indicative of a role for low dose iron in improving mood in the general population using a standardised and validated measure of mood. Additionally, as no treatment effects were observed for measures of fatigue, the previous suggestion that effects on depression were indirect effects of concomitant fatigue (Price et al., 2017) is not supported. This elucidates a potentially causal role of functional iron in dopaminergic activity as deficits in dopamine are associated with reduced D2 receptors that predict presentation of depression (Hamilton et al., 2018), However, deficits in dopamine and D2 receptors are also predictive of fatigue (Meeusen et al., 2006), stress (Campus et al., 2017) and aggressive behaviour (Chester et al., 2016), for which no effects of iron treatment were found. Iron treatment alone also unexpectedly engendered a reduction in PCS as a measure of physical health despite no significant differences in reports of adverse events between active treatment groups. These findings are inconsistent with previous findings associating iron treatment with improved physical performance and quality of life (Favrat et al., 2014; Rubeor et al., 2018), and no effects at all upon PCS (Patterson et al., 2001). Further research is therefore required to understand the potential underlying mechanisms behind such findings.

The current study unexpectedly identified no significant effects of treatment on cognitive function. Previous iron RCTs in non-anaemic women of reproductive age have found positive

treatment effects of iron on cognitive function at much higher doses of iron (60 – 260 mg elemental iron) from ferrous formulations (Bruner et al., 1996; Lambert et al., 2002; Leonard et al., 2014). This leads to the suggestion that the low dose of iron used in this study may not have been sufficient to elicit a cognitive change. However, examining the data by treatment group does not account for individual variation in response to treatment, which may lead to inaccurate conclusions regarding the true impact of iron supplementation (Murray-Kolb & Beard, 2007). This is especially appropriate as the study observed some significant improvements to haemoglobin and serum ferritin concentrations in the placebo group that were not associated with elevated CRP and vice versa for both iron treatment groups.

Although not significantly different, the observed numerical supremacy of iron and vitamin C compared to iron alone for improving iron status parameters and increasing the likelihood of serum ferritin and haemoglobin responders is consistent with suggestions of the ability of vitamin C to improve the bioavailability and absorption of iron (Chiamchanya, 2013). The lowered menstrual blood loss scores in the iron and vitamin C treatment group compared to placebo may have led to the superior iron status in this group; however, reduced menstrual blood loss may also be a consequence of such improvements to iron status. Menstrual blood loss is shown to significantly predict iron status (Harvey et al., 2005; Kocaoz et al., 2019) though previous investigations have found that iron supplementation with 60 mg elemental iron weekly or for the first four days of the menstrual cycle does not improve menstrual blood loss in IDA women of reproductive age (Bani et al., 2014). However, a multivitamin and mineral formula containing iron, vitamin C, essential amino acids and B-vitamins administered daily for 28 days to anaemic, menstruating women caused significant reductions in menstrual blood loss (Cornelli & Belcaro, 2015). The current study has extended these findings to a non-anaemic population and has reduced the number of confounding minerals and vitamins to infer a more specific causal relationship between iron and menstrual blood loss. This indicates the possibility of a beneficial cycle of improved iron status following iron and vitamin C co-supplementation leading to reduced menstrual blood flow, subsequently leading to further improved iron status. It is interesting to note though, that the efficacy of iron and vitamin C for improving iron status compared to placebo is not coupled with behavioural improvements to cognition, mood, or wellbeing. The recommended daily allowance of vitamin C for women of reproductive age in the United Kingdom is 40 mg (PHE, 2016) and supplementation with doses greater than the RDA are associated with pro-oxidant activity (Fisher & Naughton, 2004). It is reasonable to suggest that although iron and vitamin C co-supplementation is effectively increasing iron status, it may be doing so at the expense of increased oxidative stress, which may dampen the beneficial effects experienced with iron alone. Although, co-supplementation of iron and vitamin C is shown to have similar antioxidant properties in comparison to iron

alone in non-anaemic female adolescents (Khoshfetrat et al., 2013). Further investigation is thus warranted to infer a causal effect as no measures of oxidative stress were explored in this study. However, it must also be considered that baseline vitamin C concentrations across all treatment groups would be classed as depleted or marginally depleted on average according to the status limits described previously in section 4.2.7.1. Yet, following supplementation with a dose of vitamin C where plasma saturation is to be expected (Levine et al., 2001) there was no significant improvement in vitamin C status. Although standardised methods were used for vitamin C analysis with quality control measures, these unexpected findings must be interpreted with caution.

Overall, the current study addressed several limitations concerning previous RCTs including supplement dose and duration, controlling for inflammation, haematological sample and menstrual cycle timing and the previous lack of consideration for supplement bioavailability and tolerability. Consequently, the current study is the first to employ a low dose iron bis-glycinate chelate treatment that allowed for the comparison of potentially separate effects of iron alone or co-supplemented with iron enhancing vitamin C. Biochemical analyses revealed that the low dose was capable of improving both haemoglobin and serum ferritin from baseline, which demonstrates superior efficacy compared to higher doses that have only caused significant improvements in serum ferritin concentrations (Leonard, Chalmers, Collins, & Patterson, 2014; Murray-Kolb & Beard, 2007). This was also achieved in the absence of significantly greater numbers of reported adverse events compared to placebo and no significant differences in treatment compliance. Such findings provide further support for the tolerability of the iron bis-glycinate formulation. Conversely, numerically greater adverse events were reported in the placebo group compared to active treatment groups. However, an increase in the reporting of side effects may be a consequence of negative expectations held by participants of ferrous iron supplements (Tolkien et al., 2015). Negative expectations are often stimulated following the discussion of potential side effects, which may then reduce treatment efficacy, increase reports of side effects and exaggerate feelings of worry and concern (Barsky, 2017; Petrie & Rief, 2019); this is a phenomenon known as the nocebo effect. This may explain why there was no significant difference across all three treatment groups. In addition, compliance was simply calculated by pill counting, which cannot account for participants potentially discarding tablets to give the impression of adherence to treatment protocols (Jimmy & Jose, 2011; Pullar, Kumar, Tindall, & Feely, 1989) to cause overestimates of compliance.

Conversely, a potential limitation of the current study concerns baseline and post-dose blood samples collected under different conditions. Baseline samples were collected in the afternoon

in a non-fasted state whilst post-dose samples were collected in the morning in a fasted state. Haemoglobin concentrations are subject to significant postprandial reductions when compared to 12-hours fasted (Kościelniak, Charchut, Wójcik, Sztefko, & Tomasik, 2017; Lippi et al., 2010), which may confound the haematological findings. Similarly, as stated in section 4.2.7.2, zinc levels are subject to diurnal variation. Future research should ensure consistency across blood samples to account for within-day variation of haemoglobin and zinc. It must also be considered that the combination of nutritional education and supplement provision is shown to promote healthier eating habits and lifestyles amongst female populations in developed nations (Lua & Wan Putri Elena, 2012). As participants in the current study were informed of their iron status prior to starting treatment, this information may have prompted a conscious or sub-conscious change in dietary habits. Although at the post-dose assessment participants were asked if they had made any changes to their diet, it is possible that they may not actively recognised the change. Completion of the FFQ at the end of the intervention may have been able to accurately capture this information; however, smartphone applications are progressively being developed to improve the reporting of dietary intake and are widely accepted with respect to participant usability (Ferrara, Kim, Lin, Hua, & Seto, 2019). Future RCTs should consider the potential role of smartphone applications for monitoring dietary habits across long-term nutritional intervention studies. Additionally, future RCTs should consider utilising objective measures of physical activity, not only for assessing changes in physical activity level due to discrepancies between self-reported activity and objectively measured reports (Nelson, Taylor, & Vella, 2019), but to determine when participants exercised in comparison to when they took their treatment. As a single bout of endurance exercise at a moderate-vigorous intensity augments hepcidin levels between 0- and 6-hours post-exercise (Domínguez et al., 2018), this may coincide with iron supplement administration and reduce its efficacious absorption. Collecting additional data on participants during the intervention period would allow further refinement surrounding the chronic effects of iron supplementation.

Regarding the population sampled, future research should consider the influence of medical history and genetic influences. Early iron deficiency invokes long-term irreversible neurological effects that are not amenable to iron supplementation (Lozoff et al., 2006). It may therefore be imperative for future research to consider screening participants based on medical history to account for diagnoses of early iron deficiency. Additionally, iron status is not only influenced by environmental and physiological factors; serum transferrin genetic variants reduce iron transport to the tissues increasing the risk for iron deficiency (Blanco-Rojo et al., 2011). Iron intervention studies have observed significant increases in iron status in iron deficient women of reproductive age, except for those with the minor allele of single nucleotide polymorphism

rs3811647 (Blanco-Rojo et al., 2010). Both global and European prevalence of this rs3811647 polymorphism is estimated at 34 % ("rs3811647 RefSNP Report - dbSNP - NCBI", 2020); of the 56 NAID women included in the analyses for the current study, ~ 19 women may have had the polymorphism. Such genetic polymorphisms surrounding iron metabolism demonstrate significant associations with decreased serum ferritin (Ji, Flower, Hyland, Saiepour, & Faddy, 2018; Sørensen et al., 2012), thus greater iron doses may be required to elicit psychological and physiological benefits. This suggests the potential for incorporating nutrigenomics into future iron RCTs to further clarify the benefits of iron supplementation in women of reproductive age.

The findings of the current study provide novel evidence of the efficacy of a low dose iron bis-glycinate chelate supplement for significantly improving iron status, highlighting the absorptive and tolerability benefits associated with the bis-glycinate formulation regardless of vitamin C co-supplementation. However, the lack of findings regarding cognitive and behavioural function imply that the iron dose may indeed be too small compared to previous iron RCTs to have an effect in non-anaemic women of reproductive age. Future iron RCTs focussing on NAID women of reproductive age should consider lower doses of vitamin C, objective measures of physical activity, medical history, genotypes, time of blood sampling and monitoring dietary habits over the intervention period. Overall, the findings suggest that 16 weeks supplementation of iron bis-glycinate chelate is effective for improving iron status, depression, total mood disturbance and menstrual blood loss with minimal associated adverse events in non-anaemic women of reproductive age.

Chapter 5 **THE EFFECT OF IRON BIS-GLYCINATE CHELATE AND VITAMIN C CO-SUPPLEMENTATION ON CEREBRAL BLOOD FLOW AND ENERGY METABOLISM AT REST AND DURING COGNITIVE DEMAND IN WOMEN OF REPRODUCTIVE AGE**

5.1 Introduction

To produce a change in cognitive and behavioural functioning, a change in neural function is required to drive this action. In the adult brain, increases in neural activity evoke increases in the demand for delivery and consumption of metabolic resources, namely oxygen and glucose, for ATP production (Vazquez et al., 2010). Consequently, cerebral blood flow (CBF) is readily augmented to satisfy the demand for tissue oxygen; a mechanism referred to as neurovascular coupling (Kozberg & Hillman, 2016). However, a paradoxical relationship exists as the delivery of oxygen through increased CBF exceeds the cerebral metabolic rate of oxygen and ATP consumption (Leithner & Royl, 2014; Uludag et al., 2004). It is proposed that although the CBF response to neural activation may not be wholly necessary, it has evolved to protect brain function and thus cognitive performance during oxygen-limiting states (Leithner & Royl, 2014). This is especially true as although the brain only comprises a small fraction of human total body mass, it is the largest source of energy consumption with neurons consuming 75 to 80 % of energy produced in the brain (Hyder, Rothman, & Bennett, 2013). Brain imaging techniques rely on measuring cerebral haemoglobin and the diminished oxygen-carrying capacity of erythrocytes in IDA because of reduced systemic haemoglobin may lead to the assumption of IDA-associated reductions in cerebral haemoglobin. However, anaemic hypoxia is induced in IDA, which evokes increases in cardiac workload to ensure delivery of oxygen to the tissues (Pereira & Sarnak, 2003). In IDA infants, this adaptation is shown to contribute to increasing CBF velocities alongside increases in nitric oxide for augmented vasodilation as a neuroprotective compensatory mechanism to ensure sufficient oxygen delivery for neuronal activity (Aliefendioglu et al., 2007; Hare, 2004). However, independent of haemoglobin concentrations, NAID impairs cardiomyocyte function by provoking a hypoxic response that causes mitochondrial dysfunction (Hoes et al., 2018); this infers a reduction in ATP and an impaired ability of cardiomyocytes to contract and relax. Concurrently, NAID is associated with an increased blood viscosity (Broberg et al., 2006; Khaled et al., 1998) potentially because of iron deficiency-associated thrombocytosis and red blood cell aggregation (Evstatiev et al., 2014; Khaled et al., 1998), and is accompanied by symptoms such as irritability, headaches and exercise intolerance (Fairbank, 2001). However, despite these observed effects of iron on cerebrovascular parameters, studies investigating the effects of iron status on CBF are limited to IDA infants (Aliefendioglu et al., 2007), whilst the effects of iron supplementation on CBF are yet to be investigated in any population.

Few studies have investigated the impact of iron on functional outcomes of neural activity and changes in cognitive functioning. Observational studies using electroencephalography (EEG) have identified impairments to N200 and P300 event-related potential (ERP) latencies and P200 and P300 ERP amplitudes in IDA men and women in comparison to their IS counterparts (Khedr et al., 2008). Shorter ERP latencies and increased ERP amplitudes are associated with superior cognitive performance (Sur & Sinha, 2009) and as P300 latency and amplitude were in turn, negatively and positively associated with haemoglobin, serum ferritin and total IQ, a role for iron in brain function may be inferred. Similarly, over half of IDA participants had abnormal EEG findings, which may be indicative of iron's key role in axonal growth and synaptogenesis causing brain activity alterations (Beard, 2003). These findings have been extended to a NAID population of women of reproductive age where lower levels of serum ferritin were predictive of smaller increases in θ- and γ-band power from baseline (Wenger, DellaValle, et al., 2019), indicative of a lesser ability to adapt to increases in cognitive demand (Fairclough, Venables, & Tattersall, 2005) and to modulate visual information processing and attentional perceptual mechanisms (Müller, Gruber, & Keil, 2000), respectively. Metabolic outcomes were also assessed by indirect calorimetry; a non-invasive technique that measures expired pulmonary air to determine oxygen uptake and carbon dioxide production. This provides an estimate of whole-body energy metabolism that is sufficiently sensitive to detect subtle differences in cognitive tasks requiring identical response demands (Al-Naher, Schlaghecken, Barber, & Kumar, 2016). Non-anaemic iron deficiency was shown to impair energy expenditure with lower levels of serum ferritin predicting smaller increases in respiratory rate, heart rate and energy expended with increased cognitive demand (Wenger, DellaValle, et al., 2019). Serum ferritin and haemoglobin were identified to moderate the relationship between cognitive demand and brain activity and brain activity and energy expended, respectively (Wenger, DellaValle, et al., 2019). Diminished brain activity is a proposed consequence of iron deficiency disrupting neurotransmitter regulation through inefficient energy metabolism in the brain following iron deficiency-induced mitochondrial dysfunction (Rao et al., 2003), potentially reducing the ability to handle higher cognitive loads. Similarly, mitochondrial dysfunction not only reduces aerobic respiration and ATP generation but infers a reduction in haem for oxygen transport (Paul et al., 2017) thus reducing the metabolic resources available to support the increases in brain activity following increased cognitive demand. Though, as this was a population of NAID women with non-anaemic haemoglobin concentrations, the effects due to haemoglobin are thought to be indicative of the status of an individual's energetic reserves and the level of energetic resources needed to support increases in cognitive demand (Wenger, DellaValle, et al., 2019). However, as the measures previously used for oxygen consumption are not specific to the brain, further

refinement is required to be able to associate changes in cognitive function and energy expenditure specifically to the brain.

Investigations of changes in systemic iron and functional outcomes to infer a causal relationship are similarly limited. Brain activity and cognitive performance have been assessed in women of reproductive age with serum ferritin < 20 µg/L administered iron bio-fortified beans for 18 weeks. A significantly greater change in α-band power and γ-band power across tasks of attention and visual memory, respectively, were exhibited by the iron bio-fortified group compared to the control group (Wenger, Rhoten, et al., 2019). However, participant's haemoglobin was > 90 g/L, resulting in a combined sample of both IDA and NAID participants according to worldwide haematological guidelines (World Health Organization, 2011a). This restricts the specificity of the findings, making inferences regarding the effect of iron supplementation upon brain activity in NAID difficult. Iron supplementation is also shown to benefit physical energetic efficiency and aerobic capacity of NAID female athletes (Burden, Morton, et al., 2015; Fiddler, Seymour, Hernandez-Cordero, Campos, & Haas, 2019), however metabolic measures have not been used to determine the impact of iron supplementation on NAID women of reproductive age from the general population during cognitive demand. The use of indirect calorimetry for assessing the metabolic consequences of nutritional supplements during cognitive demand is relatively novel, however acute multi-micronutrient supplementation in healthy women of reproductive age caused a dose-dependent increase in total energy expenditure and fat oxidation during cognitive task performance of graded difficulty whilst chronic supplementation over 8 weeks sustained the increase in total energy expenditure only (Kennedy et al., 2016). Acute and chronic (28 days) supplementation of a similar multi-micronutrient supplement also resulted in increased total energy expenditure for both males and females compared to placebo during cognitive demand (Dodd et al., 2020). However, the former study also utilised continuous-wave near infrared spectroscopy (CW-NIRS) to assess changes in cerebral metabolic activity during cognitive performance to quantify the capacity of multi-micronutrients to modulate whole body metabolism and metabolic substrate utilisation. After an acute dose of the multi-micronutrient supplement, total haemoglobin and oxygenated haemoglobin were increased throughout the post-dose cognitive tasks; an augmented haemodynamic response may be a consequence of improving neurovascular coupling to direct cerebral blood flow to sites of neural activity. However, CW-NIRS is limited to only assessing relative changes in cerebral haemodynamics and cannot determine absolute, quantifiable concentrations of haemoglobin as it assumes a homogenous degree of light scatter amongst the tissues (Jones, Chiesa, Chaturvedi, & Hughes, 2016). The CW-NIRS relative change data is baseline-adjusted to the concentration immediately prior to the first data point in the recording session. Therefore, CW-NIRS data is unable to quantify

gross changes in haemodynamic parameters that occur between two separate recording sessions over a chronic time-period. To provide a more accurate measure, previous studies utilising the CW-NIRS have adopted an approach that subjects the data to a second baseline adjustment by creating 'change from baseline' data using resting CW-NIRS data collected prior to the consumption of nutritional supplements on each day of data capture (Kennedy et al., 2016; Wightman et al., 2015). However, frequency domain NIRS (FD-NIRS) can determine absolute optical path-length travelled by the light to provide absolute concentrations of haemoglobin and brain tissue oxygen saturation (Jones et al., 2016). The two NIRS systems have demonstrated good agreement with one another, with no significant differences observed when monitoring cerebral haemodynamics and oxygenation (Davies et al., 2017; Fantini & Sassaroli, 2020). As this is a novel area in the field of iron deficiency and supplementation research, utilising both NIRS techniques would offer advantages for assessing the cerebral haemodynamics associated with iron status and supplementation to further knowledge regarding energy metabolism and neural activity. Utilising FD-NIRS will allow for the quantification of absolute concentrations of haemoglobin at rest to determine chronic changes following iron supplementation, in addition to any differences in absolute values between iron status groups at baseline. Any significant differences in absolute values can then be considered when analysing relative changes in cerebral haemodynamics during cognitive demand. It is essential to assess metabolic parameters alongside this as measuring cerebral haemodynamic responses alone only reflects cerebral metabolic activity and does not quantify the extent of energy expenditure change during cognitive performance (Al-Naher et al., 2016).

Overall, there is evidence to suggest adverse effects of low iron status, including NAID, on measures of cerebral blood flow, energy metabolism and cognitive function. However, to date, no study has investigated such measures simultaneously in a NAID population. Additionally, the parallel effects of iron supplementation on cerebral haemodynamics and energy metabolism to examine the capability of iron to modulate whole body energy expenditure and utilisation of metabolic substrates during increasing neural demand have not been investigated. Therefore, the present study aims to investigate the effects of 16-weeks supplementation with either 28 mg/d of iron bis-glycinate alone, iron bis-glycinate plus 240 mg ascorbic acid or matched placebo on energy metabolism and absolute and relative changes in cerebral haemodynamics during rest and increased cognitive demand in NAID and IS women of reproductive age.

5.2. Methods

5.2.1 Design & ethics

This study employed a randomised, placebo-controlled, double-blind, stratified groups design, with participants randomly assigned to one of three treatment groups (section 4.2.3). The study received ethical approval from Northumbria University's Psychology Department and was conducted according to the Declaration of Helsinki (1964). The study was registered on www.clinicaltrials.gov under the identifier NCT04477018.

5.2.2 Participants

Seventy-eight female volunteers aged 18-49 were recruited into the study as a sub-sample of those already enrolled into the study described in Chapter 4 and met the inclusion criteria outlined in sections 2.2.2 and 4.2.2[12]. Of the seventy-eight enrolled into the study, seventy completed all requirements. This sample size was related to the calculation conducted for the study described in Chapter 4 and powered for the observation of significant effects on brain activity and energy expenditure following the significant effects observed with EEG and indirect calorimetry (ICa) in a previous RCTs (Wenger, DellaValle, et al., 2019; Wenger, Rhoten, et al., 2019).

5.2.3 Physiological measures

5.2.3.1 Near-Infrared Spectroscopy (NIRS)

NIRS is a non-invasive optical imaging technique, considered an appropriate alternative for functional magnetic resonance imaging (fMRI) due to its comparatively inexpensive cost, portability, ease of use and applicability with other physiological measures and amongst populations with a limited tolerance for a claustrophobic environment as in fMRI (Minati, Visani, Dowell, Medford, & Critchley, 2011). The data extracted from NIRS strongly correlate with fMRI changes during functional brain activation following a simple motor task (Strangman,

[12] A visual representation of the participant disposition throughout the studies that comprise this thesis can be found in Appendix V.

Culver, Thompson, & Boas, 2002) and successively this correlation prevails during cognitive tasks despite reduced cortical activation in comparison (Cui, Bray, Bryant, Glover, & Reiss, 2011).

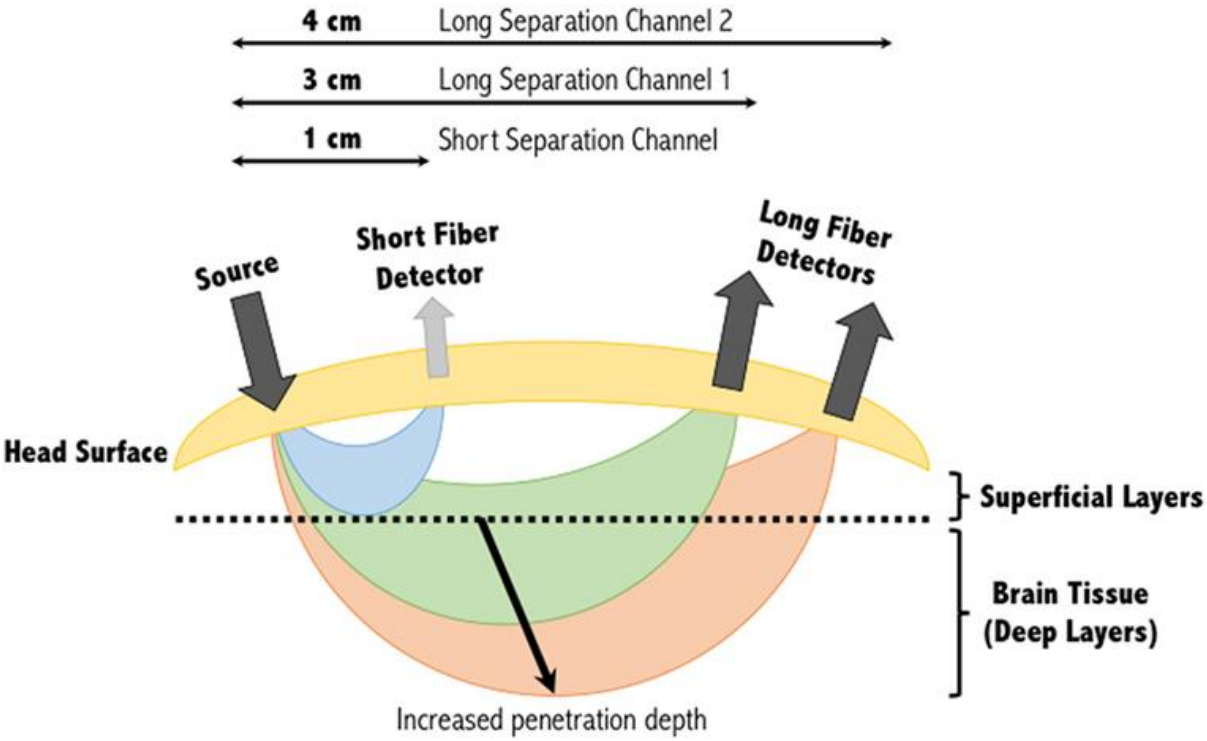


Figure 5.1 Demonstration of the typical banana-shaped curves of infrared light produced by NIRS at differing source-detector distances**.** Short separation channels of 1 cm may not allow light to sufficiently penetrate the superficial layers of the prefrontal cortex. Increasing this to 4 cm can increase light penetration to deeper layers of the prefrontal cortex (Rupawala, Dehghani, Lucas, Tino, & Cruse, 2018).

Through exploiting the differing photon absorption properties of oxygenated and deoxygenated haemoglobin chromophores, NIRS is able to quantify tissue oxygen status. Visible light (450-700 nm) cannot penetrate human tissue further than approximately 1 cm, however wavelengths along the near-infrared spectrum (700-1000 nm) can penetrate through both tissue and bone by depths of up to 8 cm for absorption by haemoglobin chromophores in the tissues (Pellicer & Bravo, 2011). NIRS technology was used in this study for measures of cerebral blood flow in the prefrontal cortex using a transmitter-receiver optode system. Infrared light is emitted from the transmitter optode following a curved pathway through the tissue of the prefrontal cortex (Figure 5.1); the light is either reflected at optical surfaces, scattered at certain tissue components or absorbed by haemoglobin chromophores. The oxygenation status of haemoglobin causes a change in the resultant spectrum, which is returned to the receiver optode (Scheeren, Schober, & Schwarte, 2012) to provide measurements of oxygenated haemoglobin (oxy-Hb), deoxygenated haemoglobin (deoxy-Hb)

and total haemoglobin (total-Hb). Cerebral blood haemodynamics were measured by two NIRS systems outlined below:

5.2.3.1.1 Continuous Wave Near-Infrared Spectroscopy (CW-NIRS)

The continuous wave Oxymon system (Artinis Medical Systems B.V.) was employed to measure relative changes in concentrations of haemoglobin in the prefrontal cortex through the absorption of infrared light. The CW-NIRS system functions by emitting light continuously at a constant amplitude, measuring only the amplitude of light decay (Strangman, Boas, & Sutton, 2002). Upon return to the receiver, the light decay denotes changes in regional cerebral blood flow by measuring change in cerebral haemoglobin (Hoshi, 2007). Concentration changes of oxy-Hb, deoxy-Hb and total-Hb are quantified using the modified Beer-Lambert Law (Kocsis, Herman, & Eke, 2006), with respect to an initial baseline value arbitrarily set to zero, adjusting the differential path length to the participant's age using the proprietorial software to account for the additional distance travelled by the infrared light due to scattering (Duncan et al., 1996; Duncan et al., 1995; Pellicer & Bravo, 2011).

Light was emitted at 764 and 858 nm and data collection recorded at a time resolution of 10 Hz. A 2-channel configuration was used (2 transmitter-receiver optode pairs) via a standard optode holder headband that ensured a distance of 4 cm between the transmitter and receiver optodes within each pair and their positioning over the left and right hemispheres of the prefrontal cortex corresponding to Fp1 and Fp2 electroencephalogram (EEG) positions of the international 10-20 system. To account for potential motion artefacts during data collection, individual tasks were separated by rest periods to endorse this as a time to change posture rather than during the tasks. Markers were inserted throughout the NIRS recording to time-stamp specific epochs corresponding to individual tasks and their repetitions. Therefore, only NIRS data collected during task completion was analysed. All quantities were measured as concentration change in micromoles per litre (µmol/L).

5.2.3.1.2 Frequency Domain Near Infrared Spectroscopy (FD-NIRS)

Progressing from CW-NIRS, FD-NIRS systems can determine absolute optical path travelled by the light to provide absolute concentrations of haemoglobin (Jones et al., 2016). This is determined by recording the intensity of light attenuation reflected onto the tissues and phase shift. Analysis of the latter allows for an accurate quantification of the total distance travelled and the subsequent degree of light scatter through the tissue (Lange & Tachtsidis, 2019). The

FD-NIRS systems have therefore progressed from the relative values of the CW-NIRS to delivering absolute values for oxy-Hb, deoxy-Hb, total-Hb and brain tissue oxygen saturation percentage (Ox%). The two systems have demonstrated good agreement with one another, with no significant differences observed when monitoring cerebral tissue saturation during increases of experimentally induced hypoxia (Davies et al., 2017).

The OxiplexTS Frequency-Domain Near-Infrared Tissue Oximeter (ISS, Inc., Champaign, IL, USA) was the dual-channel FD-NIRS system used. The instrument comprises two flexible sensors made of polyurethane rubber, within which optical fibre laser diodes are embedded and glued in pairs to four prisms (eight fibres per channel) located at 2.0-, 2.5-, 3.0- and 3.5 cm respectively from the detector bundle. Of the eight fibres per channel, four emit wavelengths of 690 nm and four emit wavelengths of 830 nm modulated at a frequency of 110 MHz. Individual right-hand and left-hand sensors were positioned on the participants' forehead so that the bottom edge was level with the top of the eyebrows and that the middle edges touched at the midline of the forehead. The sensors were secured in place by Velcro straps and a self-adhering bandage. Data was collected at 5 Hz and quantities measured in micro molars (µM).

5.2.3.2 Indirect calorimetry (ICa)

Oxygen uptake and carbon dioxide production from expired pulmonary air were measured using a portable high-resolution spiroergometry system using breath-by-breath technology (Metalyzer 3B, Cortex, Leipzing, Germany) in conjunction with the MetaSoft Studio software. The ICa system provides a non-invasive, functional analysis of whole-body energy expenditure and fuel substrate utilisation at rest and during cognitive activity, through the quantification of inhaled oxygen and exhaled carbon dioxide. In healthy participants, ICa demonstrates real-time metabolic comparisons during simple cognitive task performance through significantly greater energy expenditure during task performance in comparison to rest phases (Al-Naher et al., 2016). The use of ICa in nutritional intervention studies is relatively novel, however recent studies provide support for its application as a sensitive technique for monitoring changes in metabolic parameters following nutritional supplement administration (Dodd et al., 2020; Eschle, Goodall, Kennedy, & Wightman, 2019; Kennedy et al., 2016).

During the sampling period, participants were fitted with a mask that covered the mouth and nose; this was connected to the Metalyzer via falconia tubing allowing for pulmonary gas

analysis. Standard formulae (Frayn, 1983) were used to calculate total energy expenditure (TotalEE), respiratory exchange ratio (RER), fat oxidation (FatOx) and carbohydrate oxidation (CHOx) from the data.

5.2.4 Cognitive Tasks

The following tasks were completed in the current study:

5.2.4.1 Serial Threes Subtraction Task

One minute of the same task outlined in section 2.2.4.8.

5.2.4.2 Serial Sevens Subtraction Task

One minute of the same task outlined in section 2.2.4.8.

5.2.4.3 Rapid Visual Information Processing Task (RVIP)

One minute of the same task outlined in section 2.2.4.9.

5.2.4.4 Visual analogue scales (VAS)

Participants rated their current subjective ‘mental fatigue’ and ‘alertness’ states by placing an ‘X’ on a 100 mm line with the end points labelled “not at all” (left hand end; 0) and “extremely” (right hand end; 100).

5.2.5 Procedure

The procedures outlined in sections 2.2.8 and 4.2.8 were followed for all participants enrolled in the current study. When participants were informed of their eligibility for the study described in Chapter 4, they were also offered to partake in an additional assessment of cerebral blood flow and energy metabolism. Following completion of the procedure outlined in 4.2.8, the principal investigator discussed the requirements of the additional assessment in line with the participant information sheet previously supplied to the participant. Following informed consent, the FD-NIRS sensors were connected to the participant’s forehead for a baseline 5-minute rest measurement, whilst watching a non-stimulating video. Upon completion, the FD-

NIRS was removed and participants were then connected to the ICa system via facemask, which was adjusted to ensure there were no gaps allowing air to escape through anywhere but the analysis turbine. The CW-NIRS headband was then positioned on the participant's forehead. When proper functioning of both ICa and CW-NIRS systems were confirmed, participants sat for a further 5-minute resting baseline measurement whilst watching a continuation of the same non-stimulating video. This was followed by the completion of a 1-minute serial 3 subtraction task and a rating of mental fatigue and alertness, followed by a 1-minute rest. This was repeated three times before a 2-minute resting period. The same procedure was then repeated for the serial 7 subtraction and RVIP tasks (see Figure 5.2 for schematic depicting the cognitive battery and physiological measurements). Upon completion, participants were disconnected from the ICa and CW-NIRS systems and provided with their first bottle of allocated treatment, instructions on how to take them and a diary as detailed in section 4.2.8.

The Week 8 and 16 testing visits followed the same procedures outlined in section 4.2.8. However, following completion of the lifestyle, cognitive and mood assessments for the study described in Chapter 4 at week 16, the same protocol as the baseline assessment for the current study was completed.

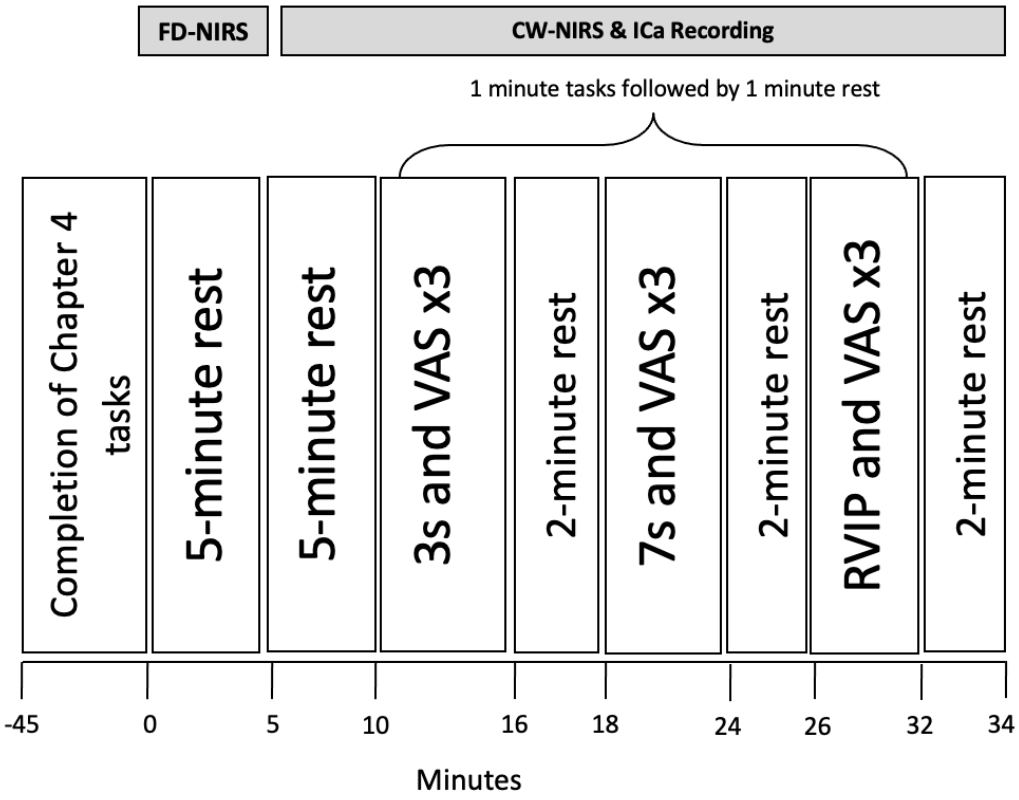


Figure 5.2 Schematic of the baseline and Week 16 assessments. Each task was completed three times for a duration of 1 minute, followed by a rating of mental fatigue and alertness and then a 1-minute rest before the next repetition of the same task. Upon completion of three repetitions of the same task, a 2-minute resting period was given prior to beginning the next series of tasks. 3s, serial 3 subtractions; 7s, serial 7 subtractions; RVIP, rapid visual information processing; VAS, visual analogue scales.

5.2.6 Data cleaning

Seventy participants completed all requirements of the study. Of the eight who did not complete the study; four were lost to follow-up; two withdrew due to time commitments; one withdrew due to self-reported lack of compliance; and one withdrew due to pregnancy. Of those who completed the study, 35 participants were IS and 35 were NAID at baseline. The per-protocol analysis excluded three participants for elevated CRP (>10 mg/L); two for a treatment compliance below 80 %; one for elevated serum ferritin in the absence of inflammation (> 150 µg/L confers a risk of iron overload in the general population (World Health Organization, 2011b)) and one for haemoglobin outside of the normal range of 120-150 g/L (Lewis et al., 2004).

Prior to conducting the analyses, the physiological and cognitive raw data was extracted and cleaned following the same procedures outlined in section 2.2.9 and 4.2.9 for the removal of anomalous and outlier data. One data set was removed from FD-NIRS analyses due to missing post-dose performance data following equipment malfunction. One data set was removed from CW-NIRS analyses due to missing baseline performance data following data catchment errors. One data set was removed from ICa analyses due to missing baseline data following data catchment errors. One data set was also removed from the cognitive and behavioural data analyses due to missing baseline performance data following data catchment errors. The final data sets for all physiological, cognitive and behavioural analyses each consisted of a sample of N = 62.

5.2.7 Statistical methods

Baseline differences across iron status and treatment groups were investigated using one-way ANOVAs. Subsequently, data were analysed using linear mixed models. Separate linear mixed models were conducted for each outcome to investigate main effects of treatment (placebo, iron, iron and vitamin C), with treatment appearing as a fixed factor in the models.

5.2.7.1 Near-Infrared Spectroscopy (NIRS) data

The Frequency Domain NIRS (FD-NIRS) raw data [oxygen saturation % (Ox%), total haemoglobin (THb), oxygenated haemoglobin ($Hb0_2$) and deoxygenated haemoglobin (Hb)] were extracted and averaged over the 5-minute resting period. To investigate the main effect of treatment, treatment group (placebo, iron, iron and vitamin C) was included as a fixed factor in the models and respective baseline average values were entered as a covariate. In the first instance, FD-NIRS data were analysed with hemisphere as a factor (left, right). As no treatment-related effects of hemisphere were found, the data was averaged across the hemispheres for analysis. Additionally, baseline serum ferritin was included as a covariate in the model to account for the effect of baseline iron status on any treatment effects.

All Continuous Wave NIRS (CW-NIRS) [oxygenated haemoglobin ($Hb0_2$); deoxygenated haemoglobin (Hb) and total haemoglobin (THb)] raw data were converted to change from baseline (the baseline being the average of the 5-minute resting CW-NIRS baseline) and averaged into epochs (10 readings per second averaged into task specific lengths). As with the FD-NIRS, CW-NIRS data were initially analysed with hemisphere (left, right) as a factor but as no effects were found, averages across the hemisphere were used for analysis. The

covariance matrix structure was selected based on the structure that produced the lowest Schwarz's Bayesian Information Criterion (BIC) to indicate the best fitting model for the data (Mohammed, Naugler, & Far, 2015). Consequently, an autoregressive covariance structure was used for all models. To investigate the main effect of treatment, fixed effects appearing in the models consisted of treatment group (placebo, iron, iron and vitamin C) and epoch (1-9). Subject was also included in all models as a random factor and baseline average values and serum ferritin were included as covariates.

5.2.7.2 Indirect Calorimetry (ICa) data

The ICa raw data [carbohydrate oxidation (CHOx); fat oxidation (FatOx); respiratory exchange ratio (RER) and total energy expenditure (TotalEE)] were extracted and divided into resting and active periods. The resting data was averaged over the 5-minute rest and analysed using the same linear mixed model procedure described previously. To investigate the main effect of treatment, treatment group (placebo, iron, iron and vitamin C) was included as a fixed factor in the models and respective baseline average values were entered as a covariate. Serum ferritin was also included as a covariate to account for the effect of baseline iron status on any treatment effects.

Active ICa data was averaged into epochs (1 reading per second averaged into task specific lengths). The data were analysed using the same linear mixed model procedure described previously with CHOx, RER and TotalEE using an autoregressive covariance structure and FatOX using an identity covariance structure. To investigate the main effect of treatment, fixed effects appearing in the models consisted of treatment group (placebo, iron, iron and vitamin C) and epoch (1-9). Subject was included in all models as a random factor and respective baseline average values were entered as a covariate. Serum ferritin was also included as a covariate to account for the effect of baseline iron status on any treatment effects.

5.2.7.3 Cognitive data

The cognitive data consisted of the baseline and post-dose total responses for the serial 3 and 7 subtractions, serial 3 errors and serial 7 errors, RVIP accuracy %, RVIP correct reaction time (msec) and RVIP false alarms from all 3 repetitions of the associated tasks. The data were analysed using the same linear mixed model procedure described previously with an identity covariance structure for all models. To investigate the main effect of treatment, fixed effects appearing in the models consisted of treatment group (placebo, iron, iron and vitamin

C) and repetition (1-3). Subject was included in the models as a random factor and respective baseline scores were entered as a covariate. Serum ferritin was also included as a covariate to account for the effect of baseline iron status on any treatment effects.

5.2.7.4 Subjective mental fatigue and alertness

The subjective mental fatigue and alertness data consisted of the baseline and post-dose ratings from all 3 repetitions of the serial 3 subtractions, serial 7 subtractions and RVIP tasks. The data were analysed using the same linear mixed model procedure described previously with all models using an autoregressive covariance structure. To investigate the main effect of treatment, fixed effects appearing in the models consisted of treatment group (placebo, iron, iron and vitamin C) and epoch (1-9). Subject was included in all models as a random factor and respective baseline ratings were entered as a covariate. Serum ferritin was also included as a covariate to account for the effect of baseline iron status on any treatment effects.

5.3 Results

5.3.1 Participants

The population for analysis consisted of 31 participants in the IS group and 32 in the NAID group. Participant disposition through the trial can be found in Figure 5.3 and participant demographics for all who completed the study within each treatment group can be found in Table 5.1.

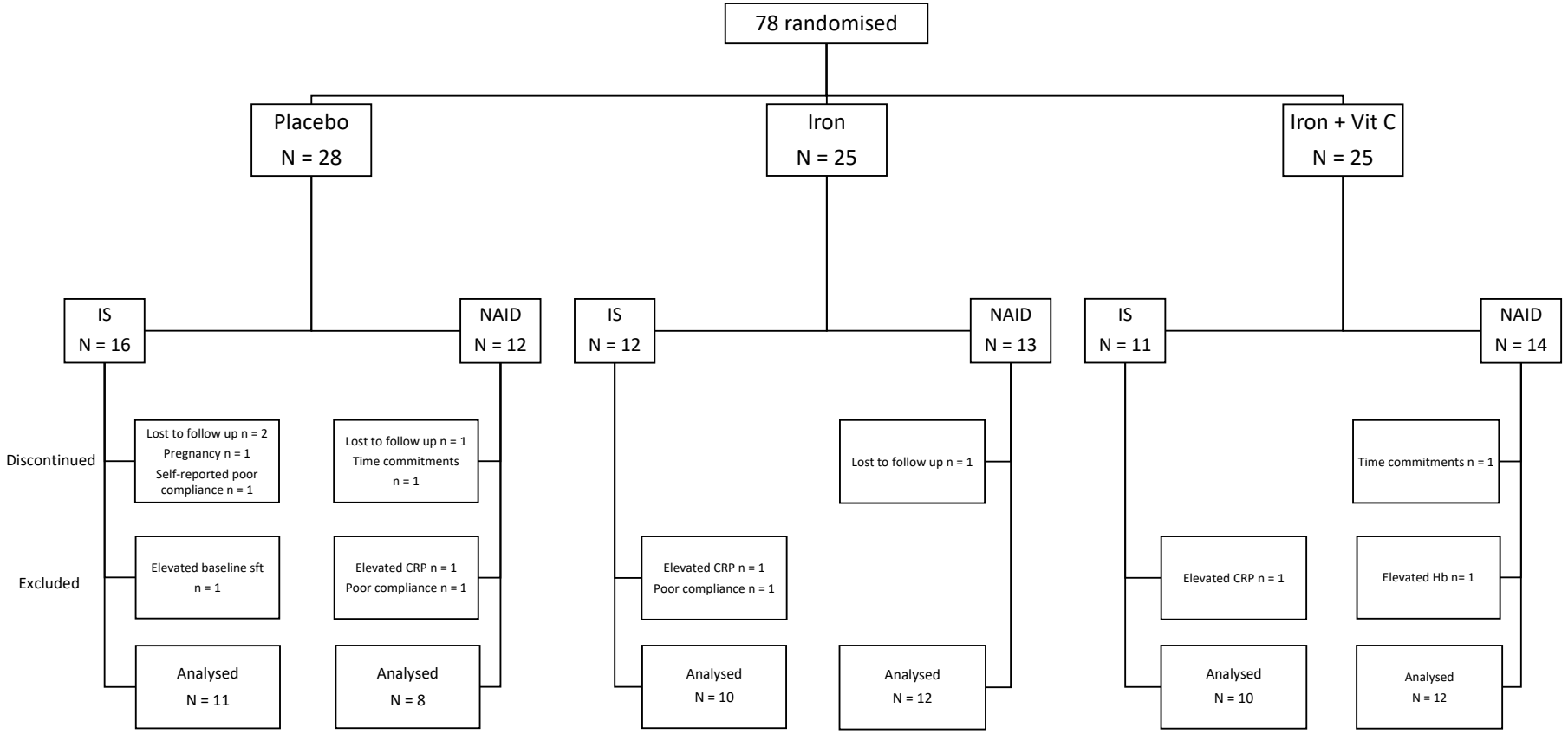


Figure 5.3 Participant disposition throughout the trial. Figure depicts the final disposition of participants throughout the study, culminating in N = 63 of the 78 randomised. IS = iron sufficient; NAID = non-anaemic iron deficient; CRP = C-reactive protein; Hb = haemoglobin; sft = serum ferritin

Table 5.1 Participant demographic information and baseline characteristics. Means and Std. Deviation (SD) are presented with F and p values of the main effects from the one-way ANOVAs conducted on the baseline data by treatment group.

		N	Baseline		Main effect	
			Mean	SD	*F*	*p*
Haemoglobin (g/L)	Placebo	19	126.05	4.56	2.93	.061
	Iron	22	127.86	5.77		
	Iron + Vit C	22	123.95	5.57		
Serum ferritin (µg/L)	Placebo	19	38.42	30.24	.420	.659
	Iron	22	34.18	28.72		
	Iron + Vit C	22	30.68	21.63		
Vitamin C (µmol/L)	Placebo	12	16.31	11.77	.505	.607
	Iron	14	19.81	12.96		
	Iron + Vit C	20	16.79	5.62		
Zinc (µmol/L)	Placebo	13	10.84	1.90	.024	.976
	Iron	15	10.68	1.98		
	Iron + Vit C	13	10.78	2.00		
Age (years)	Placebo	19	27.21	7.86	.452	.638
	Iron	22	28.91	10.26		
	Iron + Vit C	22	26.55	6.86		
Years in education	Placebo	19	17.84	3.58	1.43	.248
	Iron	22	16.27	2.10		
	Iron + Vit C	22	16.95	3.12		
BMI (kg/m^2)	Placebo	19	23.13	3.13	1.85	.166
	Iron	22	24.74	4.26		
	Iron + Vit C	22	25.25	3.36		
Systolic BP (mmHg)	Placebo	19	112.84	10.90	2.86	.065
	Iron	22	120.66	11.76		
	Iron + Vit C	22	117.41	8.47		
Diastolic BP (mmHg)	Placebo	19	76.08	7.38	1.37	.261
	Iron	22	79.59	8.24		
	Iron + Vit C	22	79.59	7.45		
Menstrual blood loss score[13]	Placebo	15	**2.45**	**1.31**	**5.27**	**.009**
	Iron	18	**5.03***	**2.89**		
	Iron + Vit C	17	4.22	2.28		
Physical activity (MET minutes)	Placebo	19	3079.26	1652.37	1.43	.249
	Iron	22	4278.68	3133.03		

[13] Menstrual blood loss was only calculated for those with a menstrual cycle; hence the reduced N. Higher scores are indicative of increased menstrual blood loss.

	Iron + Vit C	22	3354.93	2119.42		
Cereals and cereal products (g/day)	Placebo	19	249.72	99.60		
	Iron	22	231.00	119.52	.704	.499
	Iron + Vit C	22	213.12	70.46		
Dietary iron (mg/day)	Placebo	19	10.87	3.30		
	Iron	22	10.45	3.05	.132	.877
	Iron + Vit C	22	10.87	3.07		
Fish and fish products (g/day)	Placebo	19	31.28	27.04		
	Iron	22	31.84	28.10	.019	.981
	Iron + Vit C	22	30.20	30.04		
Meat and meat products (g/day)	Placebo	19	110.87	63.88		
	Iron	22	82.90	45.34	1.69	.193
	Iron + Vit C	22	82.82	56.95		
Nuts and seeds (g/day)	Placebo	19	16.18	28.42		
	Iron	22	9.61	13.19	1.50	.231
	Iron + Vit C	22	6.56	7.58		
Vegetables (g/day)	Placebo	19	276.60	164.57		
	Iron	22	323.54	160.39	.917	.405
	Iron + Vit C	22	350.94	199.82		
Alcohol consumption (units/day)	Placebo	19	0.94	1.35		
	Iron	22	0.86	0.95	.089	.915
	Iron + Vit C	22	0.80	0.81		
Caffeine consumption (mg/day)	Placebo	18	182.82	145.03		
	Iron	22	248.56	212.07	.703	.499
	Iron + Vit C	22	218.91	153.13		

* = significant difference between placebo and active treatment group below $p < .05$

5.3.2 Baseline comparisons across iron status groups

5.3.2.1 Resting FD-NIRS

No significant differences were identified across iron status groups. See Table 5.2 for means and standard deviations.

Table 5.2 Resting FD-NIRS analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups. Baseline mean scores and standard deviation (SD) are presented with F and p values for the separate one-way ANOVAs conducted on the baseline data by iron status group.

		Baseline			Main effect	
		n	Mean	SD	*F*	*p*
Ox (%)	NAID	31	66.61	5.07	.061	.806
	IS	28	66.33	3.41		
THb (µM)	NAID	31	39.85	7.27	.207	.651
	IS	30	40.71	7.56		
HbO_2 (µM)	NAID	29	26.01	5.15	.000	.990
	IS	31	25.99	5.52		
Hb (µM)	NAID	31	13.25	3.02	.041	.840
	IS	28	13.40	2.45		

Ox, oxygen saturation; THb, total haemoglobin; HbO_2, oxygenated haemoglobin; Hb, deoxygenated haemoglobin.

5.3.2.2 Active CW-NIRS

A significant effect of baseline iron status was identified for total Hb [F (1, 58.85) = 4.54, p = .037]. Participants who were NAID had higher total Hb levels (1.37) than IS participants (0.62) during cognitive demand. See Table 5.3.

Table 5.3 Active CW-NIRS analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups by epoch. Baseline mean scores and standard error (SE) are presented with F and p values of the main and interaction effects.

		Baseline				Main effect	
		n	Mean	SE		*F*	*p*
Oxy-Hb	NAID	29	1.61	0.27	Iron status	1.77	.188
(µmol/L)	IS	28	1.08	0.29	Iron status*epoch	1.28	.252
Deoxy-Hb	NAID	26	-0.20	0.10	Iron status	2.09	.154
(µmol/L)	IS	28	-0.41	0.10	Iron status*epoch	.781	.620
Total Hb	**NAID**	29	**1.37***	0.25	**Iron status**	**4.54**	**.037**
(µmol/L)	**IS**	30	**0.62***	0.25	Iron status*epoch	1.30	.247

* = significant difference between baseline iron status groups below p < .05.
Oxy-Hb, oxygenated haemoglobin; deoxy-Hb, deoxygenated haemoglobin; total Hb, total haemoglobin.

5.3.2.3 Resting ICa

No significant differences were identified across iron status groups. See Table 5.4.

Table 5.4 Resting ICa analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups. Baseline mean scores and standard deviation (SD) are presented with F and p values for the separate one-way ANOVAs conducted on the baseline data by iron status group.

		Baseline			Main effect	
		n	Mean	SD	*F*	*p*
CHOx (g)	NAID	31	0.10	0.04	.029	.865
	IS	31	0.10	0.06		
FatOx (g)	NAID	31	0.05	0.02	926	.340
	IS	31	0.06	0.02		
RER	NAID	31	0.83	0.04	.677	.414
	IS	31	0.82	0.05		
TotalEE (kcal)	NAID	30	0.88	0.17	.065	799
	IS	31	0.89	0.21		

CHOx, carbohydrate oxygenation; FatOx, fat oxidation; RER, respiratory exchange ratio; totalEE, total energy expenditure.

5.3.2.4 Active ICa

No significant differences were identified across iron status groups. See Table 5.5.

Table 5.5 Active ICa analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups by epoch. Baseline mean scores and standard error (SE) are presented with F and p values of the main and interaction effects.

		Baseline			Main effect		
		n	Mean	SE		*F*	*p*
CHOx (g)	NAID	31	0.12	0.01	Iron status	.379	.541
	IS	31	0.11	0.01	Iron status*epoch	.519	.842
FatOx (g)	NAID	31	0.05	0.00	Iron status	.341	.561
	IS	31	0.05	0.00	Iron status*epoch	.554	.815
RER	NAID	31	0.84	0.00	Iron status	.433	.513
	IS	31	0.83	0.00	Iron status*epoch	.649	.736
TotalEE (kcal)	NAID	30	0.91	0.01	Iron status	.045	.832
	IS	31	0.90	0.01	Iron status*epoch	.709	.684

CHOx, carbohydrate oxygenation; FatOx, fat oxidation; RER, respiratory exchange ratio; totalEE, total energy expenditure.

5.3.2.5 Cognitive performance

No significant differences were identified across iron status groups. See Table 5.6.

Table 5.6 Cognitive performance analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups by repetition. Baseline mean scores and standard error (SE) are presented with F and p values of the main and interaction effects.

		Baseline			Main effect		
		n	Mean	SE		*F*	*p*
SS3 total responses	NAID	30	19.03	1.08	Iron status	.033	.857
	IS	31	19.31	1.10	Iron status*rep	.114	.893
SS3 errors	NAID	30	0.72	0.14	Iron status	3.11	.083
	IS	31	1.06	0.14	Iron status*rep	.736	.481
SS7 total responses	NAID	31	12.39	0.77	Iron status	.501	.482
	IS	31	13.17	0.80	Iron status*rep	2.09	.129
SS7 errors	NAID	31	1.11	0.14	Iron status	.366	.547
	IS	31	0.98	0.15	Iron status*rep	2.86	.062
RVIP accuracy %	NAID	31	74.14	3.06	Iron status	.002	.966
	IS	30	74.33	3.26	Iron status*rep	.386	.681
RVIP correct RT (msec)	NAID	31	491.85	9.41	Iron status	1.35	.250
	IS	30	475.83	10.07	Iron status*rep	1.84	.164
RVIP false alarms	NAID	31	0.35	0.09	Iron status	.854	.360
	IS	30	0.47	0.10	Iron status*rep	.311	.733

5.3.2.6 Mental fatigue and alertness VAS

No significant differences were identified across iron status groups. See Table 5.7.

Table 5.7 Mental fatigue and alertness VAS analysis outcomes for non-anaemic iron deficient (NAID) and iron sufficient (IS) iron status groups. Baseline mean scores and standard error (SE) are presented with F and p values of the main and interaction effects.

		Baseline				Main effect	
		n	Mean	SE		*F*	*p*
Mental fatigue	NAID	30	40.02	3.21	Iron status	.309	.580
	IS	30	37.46	3.31	Iron status*epoch	.612	.767
Alertness	NAID	30	44.92	3.26	Iron status	.975	.327
	IS	30	40.30	3.36	Iron status*epoch	.468	.878

5.3.3 Baseline comparisons across treatment groups

5.3.3.1 Resting FD-NIRS

No significant differences were identified across treatment groups at baseline.

5.3.3.2 Active CW-NIRS

No significant differences were identified across treatment groups at baseline.

5.3.3.3 Resting ICa

A significant difference in baseline total energy expenditure was identified [F (2, 57) = 3.48, p = .038]. Post hoc comparisons revealed significantly lower energy expended by the placebo group (0.79) than the iron and vitamin C group (0.95; p = .036).

5.3.3.4 Active ICa

A significant treatment x epoch interaction was identified for carbohydrate oxidation [F (16, 245.68) = 1.70, p = .047]. Post hoc comparisons indicated that the iron group had significantly higher carbohydrate oxidation (0.12) than the placebo group (0.07; p = .035) during epoch 9 (RVIP repetition 3) at baseline.

A significant treatment x epoch interaction was identified for total energy expenditure [F (16, 249.14) = 2.21, p = .006]. Post hoc comparisons indicated that the iron group had significantly higher total energy expenditure levels than the placebo group during epochs 2 (serial 3's repetition 2) and 6 (serial 7s repetition 3) (p < .05). Additionally, the iron and iron and vitamin C groups both had significantly higher total energy expenditure levels than the placebo group during epoch 9 (RVIP repetition 3) (p < .05).

5.3.3.5 Cognitive performance

A significant treatment x repetition interaction was identified for RVIP accuracy [F (4, 100.98) = 3.00, p = .022]. However, post hoc comparisons revealed no significant differences between treatment groups across epochs.

5.3.3.6 Mental fatigue and alertness VAS

No significant differences were identified across treatment groups at baseline.

5.3.4 Treatment effects

5.3.4.1 Resting FD-NIRS

The analysis revealed no significant main effects of treatment. See Table 5.8.

Table 5.8 Resting FD-NIRS analysis outcomes for placebo, iron and vitamin C and iron only treatment groups. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main effects of treatment from the linear mixed models.

		Baseline			Post-dose		Treatment main effect	
		n	Mean	SE	Mean	SE	*F*	*p*
Ox (%)	Placebo	15	67.09	0.96	65.91	0.65	.664	.520
	Iron	18	66.50	0.89	66.71	0.56		
	Iron + Vit C	20	66.00	1.06	66.87	0.59		
THb (µM)	Placebo	17	39.77	1.74	39.47	0.92	.380	.686
	Iron	18	40.52	1.68	40.59	0.90		
	Iron + Vit C	18	40.47	1.55	39.95	0.90		
$Hb0_2$ (µM)	Placebo	16	25.07	1.03	25.34	0.88	.892	.416
	Iron	19	26.01	1.36	26.92	0.79		
	Iron + Vit C	20	26.78	1.13	26.06	0.81		
Hb (µM)	Placebo	17	12.81	0.65	13.96	0.34	.667	.518
	Iron	19	13.42	0.05	13.46	0.32		
	Iron + Vit C	20	13.64	0.69	13.52	0.31		

Ox, oxygen saturation; THb, total haemoglobin; HbO_2, oxygenated haemoglobin; Hb, deoxygenated haemoglobin.

5.3.4.2 Active CW-NIRS

The analysis revealed no significant main or interaction effects of treatment. See Table 5.9.

Table 5.9 Active CW-NIRS analysis outcomes for placebo, iron and vitamin C and iron only treatment groups by epoch. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main and interaction effects from the linear mixed models.

		Baseline			Post-dose		Main effects		
		N	Mean	SE	Mean	SE		*F*	*p*
Oxy-Hb (µmol/L)	Placebo	17	1.35	0.15	1.30	0.24	Treatment	.426	.655
	Iron	17	0.99	0.10	0.99	0.24	Epoch	.844	.565
	Iron + Vit C	17	1.71	0.12	1.10	0.24	Treatment*Epoch	.655	.836
De-oxy Hb (µmol/L)	Placebo	15	-0.20	0.04	-0.28	0.09	Treatment	.074	.929
	Iron	14	-0.31	0.05	-0.33	0.09	Epoch	3.35	.001
	Iron + Vit C	16	-0.39	0.04	-0.30	0.10	Treatment*Epoch	1.24	.237
Total-Hb (µmol/L)	Placebo	16	0.81	0.13	1.05	0.20	Treatment	1.65	.202
	Iron	17	0.67	0.09	0.54	0.20	Epoch	2.25	.026
	Iron + Vit C	17	1.39	0.11	0.72	0.19	Treatment*Epoch	.996	.463

Oxy-Hb, oxygenated haemoglobin; deoxy-Hb, deoxygenated haemoglobin; total Hb, total haemoglobin.

5.3.4.3 Resting ICa

The analysis revealed no significant main effects of treatment. See Table 5.10.

Table 5.10 Resting ICa analysis outcomes for placebo, iron and vitamin C and iron only treatment groups. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main effects of treatment are presented from the linear mixed models.

		Baseline			Post-dose		Treatment main effect	
		n	Mean	SE	Mean	SE	*F*	*p*
CHOx (g)	Placebo	19	0.08	0.01	0.09	0.01	.174	.841
	Iron	20	0.11	0.01	0.10	0.01		
	Iron + Vit C	22	0.11	0.01	0.11	0.01		
FatOx (g)	Placebo	18	0.05	0.00	0.07	0.01	.671	.515
	Iron	21	0.05	0.00	0.06	0.01		
	Iron + Vit C	21	0.06	0.00	0.06	0.01		
RER	Placebo	19	0.81	0.01	0.81	0.02	.377	.688
	Iron	21	0.83	0.01	0.83	0.01		
	Iron + Vit C	22	0.82	0.01	0.82	0.01		
TotalEE (kcal)	Placebo	17	0.79	0.05	0.99	0.05	.430	.653
	Iron	21	0.90	0.03	0.97	0.04		
	Iron + Vit C	21	0.95	0.04	0.93	0.04		

CHOx, carbohydrate oxygenation; FatOx, fat oxidation; RER, respiratory exchange ratio; totalEE, total energy expenditure.

5.3.4.4 Active ICa

The analysis revealed no significant main or interaction effects of treatment. See Table 5.11.

Table 5.11 Active ICa analysis outcomes for placebo, iron and vitamin C and iron only treatment groups by epoch. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main and interaction effects of treatment from the linear mixed models.

		Baseline			Post-dose		Main effects		
		n	Mean	SE	Mean	SE		*F*	*p*
CHOx (g)	Placebo	19	0.10	0.00	0.12	0.01	Treatment	.943	.395
	Iron	21	0.13	0.00	0.15	0.01	Epoch	9.02	<.001
	Iron + Vit C	22	0.11	0.00	0.13	0.01	Treatment*Epoch	.740	.751
FatOx (g)	Placebo	19	0.04	0.00	0.05	0.01	Treatment	.389	.679
	Iron	21	0.05	0.00	0.05	0.01	Epoch	1.13	.341
	Iron + Vit C	22	0.05	0.00	0.05	0.01	Treatment*Epoch	.395	.984
RER	Placebo	19	0.84	0.00	0.84	0.01	Treatment	.830	.441
	Iron	21	0.84	0.00	0.86	0.01	Epoch	5.79	<.001
	Iron + Vit C	22	0.83	0.00	0.84	0.01	Treatment*Epoch	.643	.847
TotalEE (kcal)	Placebo	19	0.84	0.02	0.98	0.04	Treatment	.196	.823
	Iron	21	0.83	0.00	1.00	0.04	Epoch	6.93	<.001
	Iron + Vit C	22	0.90	0.01	0.96	0.04	Treatment*Epoch	.857	.620

CHOx, carbohydrate oxygenation; FatOx, fat oxidation; RER, respiratory exchange ratio; totalEE, total energy expenditure.

5.3.4.5 Cognitive performance

A significant effect of treatment for serial 3 subtraction errors was identified [F (2, 49.60) = 3.25, p = .047]. However, post hoc comparisons revealed no significant differences between the iron group (1.14; p =.984) and the iron and vitamin C group (0.68; p = .057) compared to the placebo group (1.17).

A significant effect of treatment for serial 7 subtraction errors was also identified [F (2, 50.71) = 4.27, p = .019]. Post hoc comparisons revealed significantly fewer errors made by the iron and vitamin C group (0.70) compared to the placebo group (1.30; p = .014), however no significant difference for the iron group (1.12; p = .642) compared to placebo (Figure 5.4).

See Table 5.12 for baseline and post-dose means and standard error. See Appendix X for outputs.

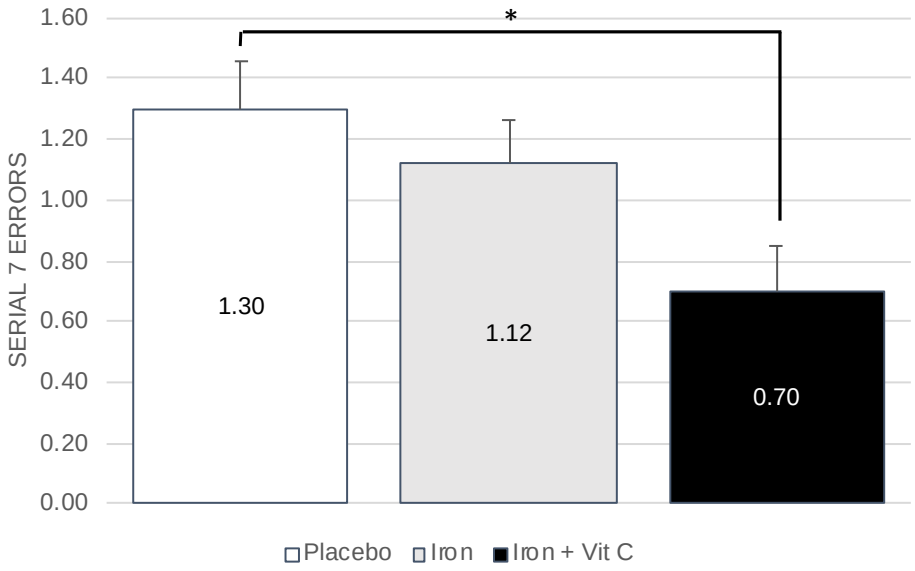


Figure 5.4 Estimated marginal means and standard error (SE) for post-dose values of serial 7 subtraction errors by treatment group (*= $p < .05$).

Table 5.12 Cognitive performance analysis outcomes for placebo, iron and vitamin C and iron only treatment groups by repetition. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main and interaction effects from the linear mixed models.

		Baseline			Post-dose		Main effects		
		n	Mean	SE	Mean	SE		*F*	*p*
SS3 total responses	Placebo	19	19.22	0.77	19.42	0.67	Treatment	1.83	.177
	Iron	22	18.90	0.87	21.12	0.62	Repetition	.916	.404
	Iron + Vit C	20	18.50	0.67	20.70	0.66	Treatment*Repetition	.543	.705
SS3 errors	Placebo	19	0.84	0.14	**1.17^{T}**	**0.16**	**Treatment**	**3.25**	**.047**
	Iron	22	0.86	0.15	1.14	0.14	Repetition	.313	.732
	Iron + Vit C	20	0.98	0.17	**0.68^{T}**	**0.15**	Treatment*Repetition	.285	.887
SS7 total responses	Placebo	19	12.75	0.59	13.26	0.37	Treatment	.709	.497
	Iron	22	13.69	0.67	13.76	0.34	Repetition	5.24	.007
	Iron + Vit C	21	12.42	0.45	13.79	0.34	Treatment*Repetition	.229	.992
SS7 errors	Placebo	19	0.82	0.13	**1.30***	**0.16**	**Treatment**	**4.27**	**.019**
	Iron	22	1.26	0.16	1.12	0.14	Repetition	.127	.881
	Iron + Vit C	21	0.98	0.15	**0.70***	**0.15**	Treatment*Repetition	1.81	.133
RVIP accuracy %	Placebo	19	78.70	2.49	70.37	3.09	Treatment	1.24	.299
	Iron	22	72.71	2.86	75.60	3.13	Repetition	1.03	.361
	Iron + Vit C	20	74.31	2.59	76.76	2.94	Treatment*Repetition	.486	.746
RVIP correct RT (msec)	Placebo	19	480.07	11.67	487.96	9.12	Treatment	.700	.502
	Iron	22	474.72	7.16	473.13	8.67	Repetition	1.01	.370
	Iron + Vit C	20	498.11	7.79	480.82	9.26	Treatment*Repetition	.763	.552
RVIP false alarms	Placebo	19	0.26	0.07	0.56	0.11	Treatment	1.02	.370
	Iron	22	0.43	0.10	0.35	0.10	Repetition	.254	.776
	Iron + Vit C	20	0.46	0.09	0.45	0.11	Treatment*Repetition	.195	.940

* = significant difference between placebo and active treatment group below $p < .05$; ^{T} = trend towards a significant difference between placebo and active treatment group below $p < .10$

5.3.4.6 Mental fatigue and alertness VAS

The analysis revealed no significant main or interaction effects of treatment. See Table 5.13.

Table 5.13 Mental fatigue and alertness VAS analysis outcomes for placebo, iron and vitamin C and iron only treatment groups by task and by repetition. Baseline raw scores, week 16 estimated marginal means and standard error (SE) are presented with F and p values of the main and interaction effects from the linear mixed models.

		Baseline			Post-dose		Main effects		
		n	Mean	SE	Mean	SE		*F*	*p*
Mental fatigue	Placebo	18	38.67	1.64	37.59	3.13	Treatment	.328	.722
	Iron	21	43.62	1.52	40.73	2.89	Epoch	5.72	<.001
	Iron + Vit C	19	34.27	1.55	38.04	3.07	Treatment*Epoch	.699	.794
Alertness	Placebo	18	40.12	1.50	43.76	2.62	Treatment	.188	.829
	Iron	21	48.62	1.65	45.35	2.43	Epoch	4.48	<.001
	Iron + Vit C	19	39.17	1.71	43.29	2.56	Treatment*Epoch	.779	.708

5.3.5 Compliance

Two participants did not return their unused treatments. For the remaining participants, compliance was observed to be very good in all three groups (102 % Placebo; 102 % Iron; 101 % Iron and Vitamin C) with a one-way ANOVA identifying no significant difference for compliance percentage by treatment group [F (2, 58) = .002, p = .998]. Fifty-eight percent of participants in the placebo group believed that had received placebo compared to 46 % and 41 % of participants in the iron and iron and vitamin C groups, respectively. Chi-squared analysis confirmed this to be a non-significant difference [χ^2 (2) = .099, p = .952].

5.4 Discussion

The current study was the first to assess the parallel effects of iron supplementation on cerebral haemodynamics and energy metabolism in a sample of NAID and IS women of reproductive age. Overall, the only baseline difference between iron status groups was an increased total haemoglobin concentration during cognitive demand for NAID participants compared to IS participants. Following 16-weeks supplementation, iron and vitamin C treatment caused a significant decrease in serial 7 subtraction errors compared to placebo, whilst this only trended towards a significant decrease for serial 3 subtractions. Finally, no significant effects of treatment were identified for cerebral blood flow parameters, ICa parameters or ratings of mental fatigue and alertness.

Concerning the significant baseline difference in total haemoglobin during cognitive demand between NAID and IS women, the finding may be useful for explaining the similarity found across these iron status groups for cognitive function, as shown in the previous chapters also. The observed increase in total haemoglobin for the NAID group may be a consequence of neurovascular coupling augmenting CBF to satisfy the demand for tissue oxygen following increased neural activity under cognitive demand. As NAID is associated with increased blood viscosity (Broberg et al., 2006; Khaled et al., 1998), vasodilation may increase as a compensatory mechanism as shown in carotid occlusion of rats (Lenz et al., 2000) and nitric oxide production may increase to allow this to occur in the same manner as in IDA female adolescents (Choi et al., 2002). This action may therefore be enhanced during cognitive demand compared to their IS counterparts to achieve the same cognitive performance. As NAID participants can achieve the same standard of performance, this may explain why there is also no significant difference between subjective ratings of mental fatigue and alertness either given the well-established relationship between cognition and mood (Baune, Fuhr, Air, & Hering, 2014). The current study has refined previous cross-sectional findings that obtained indirect estimates of brain energy through simultaneous EEG and ICa (Wenger, DellaValle, et al., 2019) by using NIRS to attribute changes in oxygen consumption specifically to the prefrontal cortex amongst NAID and IS women of reproductive age. However, contrary to such findings (Wenger, DellaValle, et al., 2019), metabolic measures of energy expenditure did not differ between the iron status groups. This may be attributed to the increased delivery of oxygen to the brain for use as a metabolic resource to fulfil the demands of increased neural activity. The study described in Chapter 2 however elucidated the issues surrounding heterogeneity when categorising continuous iron status biomarkers into iron status groups, which make comparisons between studies difficult.

Treatment effects were limited in the current study as the only significant effect pertained to a reduction in errors made on the serial 7 subtraction task and a trend for the same effect on the serial 3 subtraction task for the iron and vitamin C group. Iron supplementation (>100 mg elemental iron) has previously demonstrated significant improvements to working memory after 8-weeks in NAID women (Bruner et al., 1996; Lambert et al., 2002), however the current study extends these findings to iron and vitamin C supplementation rather than iron alone in a sample of NAID and IS women. Increased absorption and bioavailability of iron following co-supplementation with vitamin C may increase the amount of iron available for dopamine synthesis and D2 receptor functioning; processes that are critical for modulating cognitive functions within the prefrontal cortex, such as working memory (Naef et al., 2017). The low dose of iron used in the current study may have not been sufficient alone to stimulate such effects upon the dopaminergic system to observe changes in working memory accuracy. Although, systemic changes in iron biomarkers may not necessarily reflect changes in brain iron. Therefore, using fMRI for estimates of brain iron in future studies should aid in providing a more accurate interpretation of these findings. It should also be noted that the current study was not powered to detect cognitive effects and interpretations should be approached with caution.

Despite an improvement in working memory accuracy shown following supplementation with iron and vitamin C, this effect was not mirrored by changes in cerebral haemodynamics or energy expenditure. There is evidence to suggest that haemoglobin is involved in the normal physiology of the brain as it is present in dopaminergic neurons, cortical and hippocampal astrocytes and oligodendrocytes for a role in mitochondrial oxidative phosphorylation (Biagioli et al., 2009). Consequently, only measuring two channels across a small area of the prefrontal cortex in the current study limits knowledge regarding changes in cerebral haemoglobin in other areas of the brain. Haemoglobin may have increased in alternate areas of the brain to facilitate a lessened requirement for the breakdown of energy-rich fatty acids as a metabolic resource as the energy yielded from oxidative phosphorylation of glucose is used more efficiently at rest and during cognitive demand; this may then explain the null findings regarding cerebral haemodynamics. Haemoglobin is however shown to modulate the relationship between electrical brain activity and energy expenditure (Wenger, DellaValle, et al., 2019). If cerebral haemoglobin is increased in alternate areas of the brain as proposed here, future studies may benefit from utilising a multi-channel functional NIRS-EEG monitoring system (Chiarelli, Zappasodi, Di Pompeo, & Merla, 2017; Kassab et al., 2018) to determine the impact of iron supplementation upon neuronal electrical activity in addition to cerebral haemodynamics across multiple brain regions. Previous iron intervention studies regarding

NAID in female athletes (Dellavalle & Haas, 2014; Hinton & Sinclair, 2007) and non-athletes (Fiddler et al., 2019; LaManca & Haymes, 1993; McClung et al., 2009; Zhu & Haas, 1998) have observed significant benefits of iron supplementation for reducing energy expenditure and subsequently improving energetic efficiency. It must however be considered that the use of indirect calorimetry for assessing metabolic consequences of nutritional supplements is relatively novel and consequently further research within the field is required.

The current study was one of the first to investigate the parallel effects of iron status and changes in systemic iron upon functional outcomes of cognition, cerebral haemodynamics and whole-body energy metabolism following iron supplementation in a sample of NAID and IS women of reproductive age. Specifically, the employment of both FD-NIRS and CW-NIRS technology was novel to the research area and offered potential to gain insight into the biological processes that may underpin functional differences between iron status groups. However, a potential limitation of the study surrounds the cognitive tasks and their duration. The cognitive demand battery of serial 3 and 7 subtractions and RVIP is validated in literature having demonstrated sensitivity to several nutritional interventions (Kennedy et al., 2018; Kennedy, Wightman, Khan, Grothe, & Jackson, 2019; Wightman et al., 2018) including multi-vitamins and minerals (Kennedy et al., 2008; Kennedy et al., 2010), however this is when administered as a 10-minute battery of two minutes for each serial subtraction task and five minutes of RVIP. It is possible that the one-minute duration for each of the tasks was not sufficient to elicit changes in CBF in response to iron supplementation or changes in iron status. Additionally, the cognitive demand battery is designed to be repeated six times in immediate succession, accumulating to 60 minutes of cognitive demand (Kennedy & Scholey, 2004). Altogether, the shorter task durations and fewer battery repetitions may have resulted in a lack of cumulative demand. Similarly, the one-minute rest periods provided between each task repetition may not have been sufficient to allow cerebral haemodynamic responses to return to resting levels as there is evidence suggestive of the neurovascular coupling response remaining elevated for ~ 60 seconds following stimulation (Allen, Pasley, Duong, & Freeman, 2007). Consequently, cerebral responses induced following one repetition of the tasks may not have returned to resting levels before starting the next task repetition; a longer rest between repetitions, such as the 2-minutes employed when transitioning between tasks, may have been more appropriate to avoid crossover between tasks. Furthermore, the fact that significant treatment effects were identified for the serial subtractions but not for the RVIP task may suggest that only the serial subtraction tasks were amenable to iron supplementation. Although, optimal working memory performance is dependent upon an efficient mechanism of filtering irrelevant information through neural suppression to prevent overloading working memory capacity (Zanto & Gazzaley, 2009). Consequently, in line with the systematic review

described in Chapter 3 and the study described in Chapter 4 that identified no treatment effects on attention, attentional networks may already be working in an efficient manner in non-anaemic women; however, working memory systems may be using attentional networks more effectively in response to iron supplementation. Future research should consider employing working memory cognitive analogues to a treadmill test whereby the same tasks increase with difficulty in a similar manner to a previous investigation focussed upon cognitive performance, brain activity and energy expenditure (Wenger, DellaValle, et al., 2019). This is especially important as cognitive performance is facilitated by a brain-state coupled to expectations of the difficulty of an upcoming cognitive challenge (de Dreu, Schouwenaars, Rutten, Ramsey, & Jansma, 2019). As functional NIRS is limited by penetration depth that restricts detection of neural activity only from the cerebral cortex, brain areas such as the hippocampus cannot be detected. Future research could instead employ fMRI to detect hippocampal activity; however, there is evidence of associations of cortical regions such as the dorsolateral prefrontal cortex and ventrolateral prefrontal cortex, temporal and parietal cortices with successful memory encoding and retrieval (Glahn et al., 2010; Sperling, 2007; Sperling et al., 2002) in addition to the hippocampus that are detectable by functional NIRS (Jahani et al., 2017). It may therefore be possible for future research to employ either fMRI or multi-channel NIRS to detect haemodynamic changes related to working memory and episodic memory function.

The findings from the current study provide novel evidence of a low dose iron bis-glycinate chelate co-supplemented with vitamin C for significantly reducing the number of errors made on a serial subtractions task as a measure of working memory, whilst having no effects on cerebral haemodynamics or energy metabolism, compared to placebo. However, future studies powered to detect cognitive effects that use fMRI or multi-channel NIRS should be used to confidently interpret effects and attribute changes in systemic iron to changes in brain iron. Although no significant changes in cerebral haemodynamic response were observed following the intervention period, the greater levels of total haemoglobin in the prefrontal cortex demonstrated by NAID women during cognitive demand at baseline compared to their IS counterparts is a novel contribution to the research area. Further investigations should consider employing simultaneous measures of EEG, multi-channel NIRS and ICa in addition to administering a working memory task battery of incremental difficulty for longer durations with longer rest periods between task repetitions to minimise crossover effects between the tasks.

Chapter 6 GENERAL DISCUSSION

6.1 Summary of objectives

The aims of this thesis were to investigate the effects of iron status and the effects of supplementation with iron bis-glycinate chelate and iron/vitamin C on cognitive function, subjective mood, fatigue, wellbeing, and cerebral blood flow and energy metabolism in NAID and IS women of reproductive age. Investigating this population was in response to women of reproductive age largely being overlooked in favour of those considered to be at a greater risk for ID despite women's prolonged risk for ID across their reproductive years. Specifically, NAID is more prevalent than IDA among this population (Soppi, 2018; Umbreit, 2005) and few studies had directly compared associations between NAID and IS status groups concerning cognitive and behavioural function (Cook et al., 2017; Leonard et al., 2014; Murray-Kolb & Beard, 2007; Scott et al., 2017; Scott & Murray-Kolb, 2016). Moreover, as such studies categorise continuous iron biomarkers into iron status groups based upon different diagnostic criteria, heterogeneity is increased within and across samples, which may conceal the impact of ID. This is further complicated by the fact that other studies have employed continuous iron biomarkers to assess iron status (Blanton et al., 2013; Scott & Murray-Kolb, 2016). Additionally, previous cross-sectional investigations failed to account for the potential of physical activity, BMI, dietary iron and menstrual blood loss to confound measures of iron status, cognition and behavioural function. As numerous factors can determine human psychological function, it is essential to reduce the amount of noise when carrying out research at an observational level.

Previous RCTs of iron supplementation in women of reproductive age were similarly limited concerning NAID and heterogeneity surrounding the cut-offs used for NAID. Attempts to improve the efficacy, tolerability and subsequent compliance throughout the intervention periods were lacking as no supplements were considered other than ferrous formulations. Additionally, dosage of elemental iron has consistently been administered at doses much greater than the recommended daily supplementation dosage for non-pregnant, menstruating women and greater than the tolerable upper level of iron intake for gastrointestinal discomfort prevention. Consequently, a low dose iron bis-glycinate chelate either administered alone or co-supplemented with vitamin C was employed for the RCTs in this thesis in an effort to improve supplement bioavailability and tolerability and subsequently psychological and physiological outcomes. Iron bis-glycinate was chosen specifically due to the inverse relationship identified between its absorption and serum ferritin (Bovell-Benjamin et al., 2000;

Olivares et al., 1997), which suggested that its administration would be most beneficial to a NAID population compared with alternate iron preparations.

Considering the few cross-sectional and RCTs focussed on otherwise healthy, NAID women of reproductive age, further investigation was warranted to address the limitations associated and to expand the field of research. To address this, Chapter 2 aimed to investigate the associations of categorical NAID and IS status groups, and continuous iron status biomarkers haemoglobin and serum ferritin to cognitive and behavioural function, whilst controlling for the potential confounding effects of demographic and lifestyle factors. Chapter 3 aimed to systematically review the findings from RCTs investigating the effects of iron supplementation on cognition, mood, fatigue and/or well-being in menstruating, women of reproductive age. Chapter 4 comprised the first RCT of iron bis-glycinate chelate and its co-supplementation with vitamin C aimed at investigating the effect on iron status, cognitive function, subjective mood, fatigue and wellbeing in NAID and IS women of reproductive age. Finally, Chapter 5 comprised the first investigation of the effects of iron status and iron bis-glycinate chelate and its co-supplementation with vitamin C on cerebral haemodynamics and energy metabolism at rest and during cognitive demand in NAID and IS women of reproductive age.

6.2 Iron status, iron supplementation and brain function

6.2.1 Cognitive function

6.2.1.1 Iron status and cognitive function

Concerning the impact of categorical iron status on cognitive function, previous investigations have largely found no distinguishable difference in cognition between NAID and IS women (Cook et al., 2017; Leonard et al., 2014; Murray-Kolb & Beard, 2007; Scott & Murray-Kolb, 2016) despite NAID women performing worse academically compared to their IS counterparts (Scott et al., 2017). Consistent with such findings, the study presented in Chapter 2 found no associations between categorical iron status and cognitive function. When haematological parameters were treated as continuous variables, no significant associations with cognition were achieved in contrast to earlier findings (Blanton, 2013; Blanton et al., 2013; Scott & Murray-Kolb, 2016). Similarly, no differences in cognitive performance were found between iron status groups in the study described in Chapter 5. Overall, these findings are suggestive of cognition not declining in the early stages of ID.

6.2.1.2 Iron supplementation and cognitive function

The intervention study described in Chapter 4 aimed to build upon the findings from Chapter 2 to further investigate the relationship between iron and cognitive function and to determine whether iron and vitamin C co-supplementation would be more beneficial to cognition than iron alone. However, no significant effects upon cognitive function were identified. As previous iron RCTs in non-anaemic women of reproductive age have found positive treatment effects of iron on cognitive function at much higher doses of iron (60 – 260 mg elemental iron) from ferrous formulations (Bruner et al., 1996; Lambert et al., 2002; Leonard et al., 2014), this leads to the suggestion that the low dose of iron used in this study may not have been sufficient to elicit a cognitive change. However, the systematic review described in Chapter 3 highlighted the importance of accounting for individual variations in response to treatment to avoid making inaccurate conclusions regarding the actual impact of iron supplementation. This may therefore be an opportunity for further research and *post-hoc* analyses to determine whether significant changes in iron status biomarkers following supplementation result in changes in cognitive performance.

In Chapter 5, however, a significant reduction in errors on the serial 7 subtraction task and a trend for the same effect on the serial 3 subtraction task for the iron and vitamin C treatment group was discovered. This corroborated findings from previous intervention studies that observed significant improvements to working memory using much greater doses of elemental iron in NAID women (Bruner et al., 1996; Lambert et al., 2002). The positive effect of treatment is the first evidence of a low dose of iron co-supplemented with vitamin C improving cognitive performance in a sample of NAID and IS women. This may suggest that the increased bioavailability and absorption of iron when co-supplemented with vitamin C compared to iron alone allows for augmented availability of iron for dopamine synthesis and proper D2 receptor functioning, which is required for modulation of working memory performance (Naef et al., 2017). However, the study described in Chapter 5 was not powered to detect cognitive effects and as such these interpretations should be approached with caution.

Overall, the findings observed throughout this thesis suggest that cognitive function does not decline in the early stages of ID and a low dose of iron supplemented over 16 weeks is not sufficient to influence cognitive function. To improve understanding, focus should be shifted to establishing optimal haemoglobin and serum ferritin concentrations for cognitive outcomes rather than comparing NAID and IS categorical groups of women of reproductive age to avoid heterogeneity. Although a significant benefit of iron and vitamin C was observed in Chapter 5 for cognitive function, the thesis has also highlighted the potential importance of accounting

for individual variations in iron status parameters in response to treatment, which is deserving of exploration.

6.2.2 Mood & Well-being

Assessing the impact of iron status and iron supplementation on mood and well-being measures was an important aspect of this thesis due to the conflicting evidence previously presented. Chapter 2 observed no significant associations between iron status, mood and well-being, which is suggestive of no behavioural differences between NAID and IS women of reproductive age. A potential explanation for this may surround testing a sample from the general population who were otherwise healthy. Although there is evidence to suggest NAID is 15 % greater in women with depression compared to those without (Vahdat Shariatpanaahi et al., 2007), this is thwarted by the increased prevalence of mood disorders in populations from conflict-affected low-income countries as included in the study (Charlson et al., 2019). The studies that comprised this thesis however excluded those diagnosed with mood disorders, which limits the findings related to depression. More recent evidence from the United States however suggests that iron deficiency (serum ferritin < 20 µg/L) is only associated with depressive symptoms when concomitant fatigue is reported (Price et al., 2017). Consequently, independent positive associations were only identified between serum ferritin and emotional functioning and mental component summary scores as indicators of health-related quality of life. As monoamines depend upon non-haem iron for effective synthesis (Kim & Wessling-Resnick, 2014), a reduction in non-haem in the form of serum ferritin may infer emotional dysregulation through mood disturbances that are not easily detected in a non-clinical sample. As no individual associations were observed across mood measures, it may be the combination of such factors that contribute to the overall representation of emotional functioning and mental health-related quality of life in the general population.

The study described in Chapter 4, however, suggested a causal role of iron treatment alone in the aetiology of ratings of depression and total mood disturbance. Previous RCTs of iron supplementation have not observed improvements in depressive mood following higher doses of iron in NAID women of reproductive age who presented with concomitant fatigue at baseline (Vaucher et al., 2012; Verdon et al., 2003). The findings from this chapter may therefore imply that mood is amenable to a much lower dose of iron in the general population regardless of the presentation of considerable fatigue at baseline. The presentation of this effect may have also been a consequence of controlling for menstrual cycle; ensuring participants who experienced natural, or withdrawal bleeds attended their baseline and post-dose assessments

7 to 14 days following bleed onset. Controlling for the potential impact of menstrual cycle on brain activation (Pletzer et al., 2019) and emotion-related changes exhibited in the luteal phase and menses (Sundstrom Poromaa & Gingnell, 2014) may have reduced noise in this domain to allow for the detection of this effect. In contrast to this, iron treatment alone caused a reduction in PCS as a measure of physical health, which was inconsistent with previous RCTs that identified significant improvements to quality of life and physical performance following iron treatment (Burden, Pollock, et al., 2015; Favrat et al., 2014; McArthur et al., 2012; Patterson et al., 2001; Rubeor et al., 2018). Overall, the thesis has highlighted the benefit of iron for mood regulation in women of reproductive age from the general population and that further research is required to elucidate the relationship between iron treatment and physical wellbeing.

6.2.3 Fatigue

Iron deficiency is most attributed with non-haematological symptoms of weakness and fatigue, following greater reports of constant tiredness compared to those who are IS (Patterson et al., 2000). However, RCTs have largely focussed on NAID female athletes for investigations of fatigue due to their additional risk of ID with and without anaemia and its potential impact upon athletic performance through impaired aerobic capacity and increased fatigue (DellaValle & Haas, 2011; Koehler et al., 2012; Sinclair & Hinton, 2005). However, ID-associated fatigue is not limited to its impact on physical athletic performance as subjective fatigue may also present categorised by feelings of lowered energy and motivation that may subsequently increase anxious and depressive symptoms to deplete quality of life and cognitive capacity (Dziembowska et al., 2019). Chapter 2 of this thesis, however, failed to show any significant associations of iron status group, haemoglobin or serum ferritin to any of the subjective fatigue outcomes. This is consistent with findings from a meta-analysis of cross-sectional studies that failed to find associations between NAID and subjective fatigue in the general population; significant associations were only identified amongst populations with diagnosed disorders (Yokoi & Konomi, 2017). Subjective central fatigue is hypothesised to be a consequence of an elevated ratio of serotonin to dopamine, as observed in ID (Kim & Wessling-Resnick, 2014; Meeusen et al., 2006). As no individual associations were observed across mood and fatigue measures in Chapter 2, it may be that associations are not detectable in a non-clinical sample. Although their combination may affect an individuals' mental health and wellbeing through emotional dysregulation as previously evidenced (Ferreira et al., 2019) and as shown through the positive association of quality of life to serum ferritin in Chapter 2, which again may aid in explaining the Chapter 2 findings regarding fatigue, mood and wellbeing.

The systematic review presented in Chapter 3 highlighted a significant effect of iron supplementation for reducing fatigue in NAID women indicating a serum ferritin-dependent effect upon fatigue symptoms. However, the studies were subject to heterogeneity regarding the haemoglobin and serum ferritin concentration cut-offs used for classifying NAID, which left the findings open to bias. Using a serum ferritin cut-off of ≤ 20 µg/L proved most effective for improving athletic performance (Rubeor et al., 2018), and as a reduction in physical capacity often presents simultaneously with symptoms of central fatigue (Dziembowska et al., 2019) it was hypothesised that improvements to subjective fatigue may present following supplementation. However, no significant treatment effects were identified in Chapter 4 regarding subjective fatigue or sleep quality. Previous iron RCTs that have found significant effects of iron supplementation for reducing fatigue, have however used intravenous iron or much higher doses of ferrous oral formulations in clinical populations of women (Favrat et al., 2014; Krayenbuehl et al., 2011; Vaucher et al., 2012); the low dose of iron used in this study may have not been enough to initiate a significant improvement in the general population. Overall, the thesis has identified that fatigue may not be present in the early stages of ID, and that a low dose of iron is not sufficient to have a positive effect upon fatigue in a general population of non-anaemic women of reproductive age.

6.2.4 Cerebral haemodynamics

The study presented in Chapter 5 of this thesis was the first to employ NIRS to investigate cerebral haemodynamics across baseline iron status groups and to explore any changes in CBF following iron supplementation. Total haemoglobin concentrations during cognitive demand were significantly increased in NAID women compared to IS women. When considered alongside the cognitive findings in this Chapter and in Chapter 2 of no significant decline in cognitive performance in the early stages of ID, this may suggest augmented CBF acts as a compensatory mechanism for NAID women to achieve the same cognitive performance as those who are IS. Blood viscosity is increased in NAID due to impaired cardiomyocyte function following hypoxia-induced mitochondrial dysfunction (Broberg et al., 2006; Khaled et al., 1998), which stimulates increases in vasodilation as a compensatory mechanism (Lenz et al., 2000); this action may therefore be intensified during cognitive demand to satisfy the increased demand for oxygen in response to increased neural activity. However, this compensatory effect may have long-term effects; increased blood viscosity is associated with increased morbidity and mortality of several cardiovascular and cerebrovascular diseases (Naghedi-Baghdar et al., 2018), which may be exacerbated upon exhaustion of compensatory vasodilation mechanisms. Therefore, although this compensatory effect may be beneficial to NAID women in the short-term, the potential long-

term detriments it holds provide support for NAID being a clinical challenge in need of more recognition.

Conversely, no significant treatment effects on CBF were established following 16-weeks iron supplementation, indicative of CBF not being amenable to improvements in iron status parameters. Restoring iron levels through iron supplementation is shown to improve cardiomyocyte function (Hoes et al., 2018) and blood flow regulation (Halis et al., 2009) *in vitro* and in children, respectively. As this was the first study to examine the effects of iron supplementation upon CBF, the null findings are difficult to interpret and are thus deserving of further investigation, especially as only two-channels across the prefrontal cortex were measured. Employing multi-channel functional NIRS systems in future would enable a greater understanding of the effects of iron supplementation on CBF across multiple brain regions to avoid making inaccurate conclusions.

Overall, although no significant changes in cerebral haemodynamic response were revealed following 16-weeks iron supplementation, the thesis is the first to identify significant differences in CBF between iron status groups. The greater total haemoglobin concentrations in the prefrontal cortex exhibited by NAID women during cognitive demand compared to IS women provides novel insight into how compensatory mechanisms may be involved in ensuring maintenance of cognitive and behavioural function in the early stages of ID.

6.2.5 Energy metabolism

An additional aim of Chapter 5 was to investigate the effects of 16-weeks iron supplementation on energy metabolism during rest and cognitive demand in NAID and IS women of reproductive age. This was following evidence suggesting ID impairs energy metabolism with serum ferritin positively predicting respiratory rate, heart rate and energy expended as cognitive demand increases (Wenger, Rhoten, et al., 2019). However, no significant differences in measures of energy metabolism were observed between iron status groups in Chapter 5. Energy expenditure is though consistently observed to be higher during the luteal phase of the menstrual cycle (Benton, Hutchins, & Dawes, 2020; Zhang et al., 2020), which has not previously been controlled for. The null findings here may therefore have been due to a reduction in noise following controlling for where women were in their menstrual cycle for baseline and post-dose assessments. Although, when considered alongside the difference in total haemoglobin between iron status groups, the null findings may be attributable to the augmented delivery of oxygen to the brain for NAID women to fulfil the demands of increased neural activity to achieve the same performance as IS women. Therefore, the use of metabolic

resources and energy expended may not differ between iron status groups, however NAID women have more metabolic resources made available to ensure the same cognitive and behavioural outcomes are met. However, following the intervention period, no significant effects upon energy metabolism were found in contrast to previous iron RCTs (Fiddler et al., 2019; LaManca & Haymes, 1993; McClung et al., 2009; Y. I. Zhu & Haas, 1998); iron supplementation significantly reduced energy expenditure and improved energetic efficiency. To further elucidate the relationships between iron supplementation and energy metabolism, adopting an approach utilising a multi-channel functional NIRS-EEG system simultaneously with ICa may be beneficial to determine the links between neural electrical activity, cerebral haemodynamics and energy metabolism in response to improvements in iron status parameters across multiple brain regions.

Overall, this thesis included the first investigation of the impact of iron supplementation upon energy metabolism at rest and during cognitive demand in NAID and IS women of reproductive age. Although no significant effects were observed across iron status groups, when considered alongside the cerebral haemodynamics results, the null findings may be attributable to the augmented delivery of oxygen to the brain for NAID women to fulfil the demands of increased neural activity to achieve the same performance as IS women. As the use of indirect calorimetry is relatively novel in the field of nutritional interventions, further research is required in order to determine how iron supplementation may impact energy metabolism in non-anaemic women of reproductive age.

6.3 Iron bis-glycinate chelate and vitamin C

The intervention studies conducted throughout this thesis comprised the first investigations of an iron bis-glycinate chelate compound upon cognitive, behavioural, cerebral haemodynamic and energy metabolism outcomes. This was following evidence that its supplementation would allow for a more efficacious improvement of haematological measures of iron status due to increased bioavailability and tolerability (Coplin et al., 1991; Fouad et al., 2013), especially amongst a NAID population (Bovell-Benjamin et al., 2000; Olivares et al., 1997). The addition of vitamin C was also investigated as although ferrous formulations of iron supplements are more amenable to the associated benefits of vitamin C, its addition to iron bis-glycinate formulas is shown to improve the already superior efficacy (Olivares et al., 1997). The effects upon iron status that were observed in Chapter 4 were comparable as although the iron and vitamin C group had significantly higher haemoglobin and serum ferritin compared to placebo, no significant differences were identified between iron treatment groups despite numerically larger values for the iron and vitamin C group (APPENDIX IX). This was however the first

investigation of the effects of a low-dose iron bis-glycinate formula for improving iron status in NAID and IS women of reproductive age and has therefore demonstrated its ability to significantly improve iron status parameters compared to placebo with minimal associated adverse events. The findings revealed no significant benefit of co-supplementing with vitamin C, which is consistent with the biochemical analysis identifying no significant improvement in vitamin C levels for the iron and vitamin C group. A potential explanation for this could pertain to the 240 mg dose of vitamin C co-supplemented; the vitamin C RDA for women of reproductive age in the United Kingdom is 40 mg (PHE, 2016) and supplementation doses exceeding the RDA are associated with pro-oxidant activity (Fisher & Naughton, 2004), which may lead to poorer bioavailability and increased vitamin C excretion. Although the iron and vitamin C supplement was numerically superior for improving measures of iron status, the null findings between active treatments may be a consequence of the vitamin C dose increasing oxidative stress to diminish its absorptive capabilities. Iron and vitamin C co-supplementation at greater doses has been shown to have similar antioxidant properties compared to iron alone in NAID female adolescents (Khoshfetrat et al., 2013), therefore the oxidant properties of iron bis-glycinate co-supplemented with vitamin C require investigation. As ferrous formulations are far more amenable to the absorptive benefits of vitamin C, it may be beneficial for further research to measure markers of oxidative stress and to compare co-supplementation of iron and vitamin C from bis-glycinate chelate and ferrous formulations to determine the efficacy of vitamin C across supplementation methods. Overall, this thesis has provided the first evidence of the ability of iron bis-glycinate chelate to improve iron status in NAID and IS women of reproductive age with minimal associated adverse events. Although the iron and vitamin C group numerically had greater haemoglobin and serum ferritin concentrations, there was no significant advantage of co-supplementation over iron alone.

6.4 Limitations

The studies that comprise this thesis have addressed multiple limitations associated with previous cross-sectional studies and RCTs, specifically surrounding accurate determinations of iron status, the consideration of confounding variables, supplement bioavailability and tolerability. Additionally, the employment of novel methodologies to the research area, such as FD-NIRS and CW-NIRS, provided insight into the biological processes that may underpin the functional differences between iron status groups observed in this thesis and previous research. However, as discussed throughout the previous chapters, it is essential to consider methodological limitations that may have affected the findings across the studies.

Firstly, a potential issue that was overlooked in this thesis was the lack of accountability for medical history regarding iron status. There is extensive evidence suggesting that early-life IDA causes irreversible impairments to cognitive function and mood regulation that persist through to childhood (Lozoff et al., 2000; Lozoff et al., 1991), adolescence (Lozoff et al., 2000) and early adulthood (Lozoff et al., 2006; Lozoff et al., 2013) regardless of iron repletion following diagnosis. Animal model systems have identified alterations to monoamine neurotransmitter metabolism, energy metabolism, myelination (Beard & Connor, 2003; Dallman, 1986; Youdim & Green, 1978), brain iron content and brain tissue composition (Mudd et al., 2018) to be responsible for such irreversible psychological effects. Considering the Chapter 2 study, the lack of consideration for early-life ID highlights the possibility of including those classified as IS who were at a psychological disadvantage due to the neurological impairments instigated as a consequence of early-life ID. Similarly, as such psychological and physiological impairments are not amenable to iron supplementation, this may have also carried over to the RCTs reported in Chapters 4 and 5 and confound treatment effects. It may be imperative for future investigations of iron status and iron supplementation on cognitive and behavioural function to screen participants for a medical history of early-life ID and to consider excluding them on this basis to account for any neurological deficits that may confound findings.

Another methodological consideration concerns the assessment of iron status from just haemoglobin and serum ferritin. Although, the complication of concomitant inflammation for an accurate assessment of ID was addressed by measuring CRP, NAID is also hallmarked by concomitant decreases in serum iron, transferrin saturation and hepcidin and increases in total iron-binding capacity and soluble transferrin receptors (sTfR) (Sheikh et al., 2017). To determine iron status within intervention studies, serum ferritin and haemoglobin are considered sufficient for accurate assessments (Joint World Health Organization/Centers for Disease & Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, 2007). However, more recently iron status assessments have used a model of total body iron stores, which uses the log ratio of sTfR to serum ferritin to assess the full range of iron status from severe deficiency to overload (Pfeiffer & Looker, 2017). It provides a quantitative estimate as a continuous variable of the functional deficit to be corrected before iron can begin to accumulate in the body iron stores in iron deficient individuals or the size of the body iron stores when iron is present within it for those considered IS (Pfeiffer & Looker, 2017). The model has been indirectly validated by providing accurate estimations of the prevalence of ID by demographic (Cook, Boy, Flowers, & Daroca Mdel, 2005; Cook, Flowers, & Skikne, 2003; Cook, Skikne, Lynch, & Reusser, 1986), and successfully examining

prevalence of and changes in low body iron stores in response to oral iron supplementation (Cable et al., 2016; Cook et al., 2003). It is therefore recommended to measure iron stores for ID in populations more accurately than serum ferritin alone (Northrop-Clewes & Thurnham, 2013). Total body iron has already been used to determine iron status in both cross-sectional (Blanton et al., 2013; Cook et al., 2017; Scott et al., 2017; Scott & Murray-Kolb, 2016) and dietary iron intervention studies regarding cognitive function (Blanton, 2013; Wenger et al., 2017; Wenger, Rhoten, et al., 2019), which have been referred to throughout this thesis. Future cross-sectional studies and RCTs of iron bis-glycinate chelate amongst women of reproductive age should consider using the total body iron model for iron status determination.

Another potential issue that arises throughout the studies concerns the reliance on subjective measures of behaviour that could instead be measured objectively. Subjective assessments of physical activity were collected to determine baseline and post-dose levels of physical activity, however self-reported moderate to vigorous physical activity is often over-reported and sedentary time under-reported when results from the IPAQ are compared to an objective measure (Nelson et al., 2019). This may have led to inaccuracies in the confounding variables assessed in Chapter 2 as well as the effect of iron supplementation and significant responses to iron treatment upon physical activity in Chapter 4. Objective measurements of physical activity would also allow assessments of when participants exercised compared to when they consumed their treatment. Acute bouts of exercise at a moderate to vigorous intensity are evidenced to augment hepcidin levels from 0 to 6 hours post-exercise, peaking at 3 hours (Domínguez et al., 2018). This affects iron absorption in the gastrointestinal tract especially as this may coincide with iron supplement administration, thus reducing its efficacy. Additionally, serum ferritin concentrations < 30 µg/L are evidenced to blunt exercise-induced hepcidin increases (Peeling et al., 2014); iron supplementation increases the likelihood of hepcidin upregulation (Burden, Pollock, et al., 2015). Therefore, IS participants randomised to an iron treatment group with sub-optimal serum ferritin between 30-50 µg/L consuming their treatment during periods of exercise-induced hepcidin elevations may have inadvertently suppressed effective iron supplement absorption. Although no perfect tool exists for a truly accurate examination of physical activity, a systematic literature review highlights the lower levels of variability observed for validity and reliability when objective accelerometers are used compared to subjective measures specific to the behaviours of interest (Dowd et al., 2018). Future RCTs should therefore consider incorporating accelerometers that can specifically monitor timing and intensity of exercise, the data for which could be used alongside the electronic compliance applications previously discussed. Similarly, the subjective assessment of food frequency by FFQ is open to reporting bias as it requires participants to recall all foods and drinks consumed and in what quantity over the past year. This may have also led to

erroneous conclusions regarding dietary iron as a confounding variable in the study described in Chapter 2. Efforts have been made to shift from traditional pencil-and-paper based FFQs to 24-hour computer- or web-based applications to improve the accuracy of dietary recall by improving portion size estimation and minimising forgotten food items (Jobarteh et al., 2020). Additionally, smartphone applications have been created that are designed for participants to upload images of foods and meals eaten to; these can be used as a stand-alone assessment method or to enhance traditional self-report FFQ methods by reducing recall bias (Gemming, Utter, & Ni Mhurchu, 2015). Participant feedback of such smartphone applications has shown high levels of acceptance with respect to usability (Ferrara et al., 2019) and a significant preference for their use compared to completing 24-hour dietary recalls (Ambrosini, Hurworth, Giglia, Trapp, & Strauss, 2018). Future RCTs should consider the potential role of smartphone applications for monitoring dietary habits across long-term nutritional intervention studies.

Lastly, iron is a vital component in various metabolic processes in the human body but is also a micronutrient required by all living organisms promoting competition for iron for both the maintenance of human health and indigenous microbial populations. Evidence from animal studies suggests that *Lactobacillus* species possess an iron-dependent mechanism that inhibits iron absorption; the gut microbiota produces microbial metabolites 1,3-diaminopropane (DAP) and reuterin that suppress the role of the transcription factor hypoxia-inducible factor 2α (HIF-2α) for intestinal iron absorption and increasing serum ferritin (Das et al., 2020). Therefore, iron supplement absorption may be diminished in those with a gut microbiome rich in these metabolites compared to those without. Additionally, studies in iron deficient menstruating women have identified genetic variations in the transferrin gene (rs3811647) that present a reduction in iron transport to the tissues causing an increased risk for ID (Blanco-Rojo et al., 2011) with significant associations demonstrated between the minor allele of rs3811647 and decreased serum ferritin (Ji et al., 2018; Sørensen et al., 2012). Iron deficient women of reproductive age who present with the rs3811647 SNP do not significantly respond to dietary iron interventions by means of improving iron status (Blanco-Rojo et al., 2010). Adopting a metabolomics and nutrigenomics approach may be beneficial for future research to examine differences across baseline iron status groups for determining whether there are microbial or genomic differences that predispose an individual to ID and whether this can predict how an individual responds to iron supplementation.

6.5 Future research

Upon starting this thesis, research investigating the impact of iron status and iron supplementation on cognitive and behavioural function was limited in women of reproductive age, especially that concerning NAID. However, for the research field to progress it is essential to address the aforementioned limitations. Therefore, future research should consider screening for early-life IDA diagnoses, genetic polymorphisms and microbial metabolites to account for predispositions for diminished cognitive, behavioural and iron status responses; dietary habits and physical activity timing and intensity; and measuring sTfR in addition to haemoglobin and serum ferritin for the calculation of total body iron to increase the accuracy of iron status assessments. Despite the limitations discussed, the studies that comprise this thesis have contributed to the research field through investigation of a novel low-dose iron supplement and represent the first investigations of the parallel effects of iron status and supplementation upon cerebral haemodynamics and energy metabolism. Accordingly, there are several interesting findings that could be investigated further in future.

The results presented in Chapter 2 suggest that cognitive function does not decline in the early stages of ID that has previously been identified (Rebecca L. Cook et al., 2017). However, the significant improvement in working memory accuracy following iron treatment identified in Chapter 5 implies a potential role of iron in cognitive processing. To investigate these findings further, it would be appropriate to employ fMRI or multi-channel NIRS to determine whether differences in brain region activation exist during completion of working memory tasks between iron status groups and in response to iron supplementation. Additionally, to specifically determine whether deficits in brain iron exist across iron status groups it may be beneficial to employ quantitative magnetic susceptibility (QMS) as an *in vivo* indicator of brain iron content. This methodology has previously been used to confirm brain ID in restless leg syndrome patients compared to healthy controls (Li et al., 2016) and has demonstrated high specificity and sensitivity for identifying tissue iron content in cerebral grey matter regions (Langkammer et al., 2012; Lim et al., 2013; Zheng, Nichol, Liu, Cheng, & Haacke, 2013). It has also demonstrated superior efficacy for quantifying iron levels in clinical samples where changes in brain iron levels are commonly observed (Du et al., 2016; Langkammer et al., 2013). Using QMS would allow associations to be made between peripheral iron status and brain iron for a more accurate interpretation of psychological function.

However, the findings from the study described in Chapter 2 also suggest positive associations between serum ferritin and emotional functioning, yet this was not echoed in the findings of categorical iron status. This supports the notion that categorical iron status groups can be problematic and mask underlying relationships, even in the absence of excess iron. It may therefore be imperative for future studies to focus upon establishing optimum

haemoglobin and serum ferritin concentrations for cognitive and behavioural outcomes rather than comparing NAID and IS groups for non-pregnant women of reproductive age. This could involve splitting haemoglobin and serum ferritin concentrations into quantiles as in previous population studies (Murray-Kolb & Beard, 2007; Su et al., 2016), to determine optimal concentrations of haemoglobin and serum ferritin for optimal cognitive and behavioural performance. Assessing by quantile may also be more appropriate than using a fixed serum ferritin cut-off point for iron status classification considering participants whose serum ferritin may fluctuate between iron status groups across the menstrual cycle.

The findings from Chapter 4 pertaining to the low-dose iron bis-glycinate chelate formulation significantly improving haemoglobin and serum ferritin with minimal associated side effects are promising considering the evidence suggesting its conformation promotes increased iron bioavailability and supplement tolerability (Ashmead, 2001). Oral iron supplements are often attributed to adverse events due to a large proportion remaining unabsorbed and entering the colon. This may be a consequence of supplements altering the composition of the gut microbiota due to increased competition between the human host, intestinal microbes and microorganisms for iron sources (Constante, Fragoso, Lupien-Meilleur, Calvé, & Santos, 2017). Iron-fortification RCTs in African children have identified iron administration to adversely affect the gut microbiome by producing a more pathogenic gut microbiota profile and reducing beneficial commensal levels, which is associated with increased intestinal inflammation (Jaeggi et al., 2015; Zimmermann et al., 2010). Similarly, oral iron supplements are evidenced to contribute to exacerbated dysbiosis, intestinal inflammation and mucosal damage through increased oxidative stress in samples with inflammatory bowel diseases (IBD) (Carrier, Aghdassi, Cullen, & Allard, 2002; Seril et al., 2002). However, although gut iron concentrations may detrimentally influence microbiota composition, investigations concerning the chemical form of iron in murine models of IBD have identified iron bis-glycinate to have a protective role compared to alternate formulations; this was attributed to the type of iron compound administered causing significantly different clustering of microbiota communities (Constante et al., 2017). Gastrointestinal dysfunction seen in IBD and irritable bowel syndrome is frequently accompanied by comorbid psychiatric conditions (Mayer, 2000a, 2000b), which is postulated to be a consequence of disrupted communication across the gut-brain axis that modulates gastrointestinal and central nervous system functioning (Mayer, Tillisch, & Gupta, 2015). Considering the role of the microbiota as a key regulator of the gut-brain axis (Cryan et al., 2019), it seems reasonable to suggest that the beneficial effect of iron bis-glycinate formulations upon the microbiota may also affect cognitive and behavioural function. However, the influence of iron supplementation and iron bis-glycinate specifically on the gut microbiota in non-clinical adult samples from the general population remains unclear and under-explored.

The measurement of microbial communities from faecal samples pre- and post- iron bis-glycinate supplementation would allow further exploration of the potential mechanisms that underpin cognitive and behavioural function.

Finally, the null findings presented in Chapter 5 concerning cerebral haemodynamics and energy metabolism following iron supplementation are deserving of further investigation to progress the field. As haemoglobin is shown to moderate the relationship between electrical brain activity and energy expenditure and serum ferritin moderates the relationship between electrical brain activity and cognitive performance (Wenger, DellaValle, et al., 2019), employing EEG technology simultaneously with multichannel NIRS or fMRI may be a future avenue to consider. To invoke CBF effects however, it may be necessary to employ tasks of a longer duration than one-minute and to provide longer rest periods between task repetitions to account for prolonged elevations of the neurovascular coupling response following cognitive stimulation (Allen et al., 2007). As working memory tasks were the only cognitive tasks to have shown an effect following supplementation in this thesis, this may provide insight into the type of tasks that should be focussed on in future studies. As similar studies have found significant effects when a cognitive analogue to a treadmill test was employed (Wenger, DellaValle, et al., 2019), working memory tasks of increasing difficulty should be considered for future iron RCTs that aim to assess neural electrical activity, cerebral haemodynamics and energy metabolism in response to cognitive demand.

6.6 General conclusions

The aim of this thesis was to investigate the effects of iron status and co-supplementation of iron bis-glycinate chelate and vitamin C on cognitive function, subjective mood, fatigue, wellbeing, and cerebral blood flow and energy metabolism in NAID and IS women of reproductive age. The study presented in Chapter 2 revealed the first evidence of the increased prevalence of NAID compared to IDA in a general population of women of reproductive age from the United Kingdom, consistent with international epidemiological data (Soppi, 2018; Umbreit, 2005) and local estimates (SACN, 2010; PHE, 2014). The findings also provide support for cognitive function not declining in the early stages of ID in women of reproductive age, bolstering previous suggestions that only IDA may be detrimental when potentially confounding factors are considered (Cook et al., 2017). However, Chapter 2 highlighted how categorising women into iron status groups is problematic as associations present by continuous haematological parameters did not carry over to categorical iron status groups. It may be beneficial to shift focus from comparing NAID and IS groups of non-pregnant women of reproductive age, to establishing their optimum haemoglobin and serum ferritin

concentrations for cognitive and behavioural outcomes. The positive associations identified between serum ferritin and mental health-related quality of life are consistent with the known role of serum ferritin for emotional regulation (Kim & Wessling-Resnick, 2014; Perlman, Luna, Hein, & Huppert, 2014). Further investigation is however still required to account for early diagnoses of ID in infancy that may affect cognitive and behavioural findings, and employing neuroimaging methods to assess brain iron levels, neural activity, and tissue morphology to confirm theories regarding compensatory mechanisms associated with NAID.

The Chapter 3 systematic review provided an updated overview of findings from RCTs investigating how iron supplementation affects cognition, mood, fatigue and/or well-being in women of reproductive age of varied baseline iron status. Significant improvements to attention, memory, learning, fatigue, mood and well-being were observed, however the review highlighted a lack of consideration for supplement dose, duration, bioavailability and tolerability. Consequently, Chapter 4 investigated the effects of 16 weeks low-dose iron bis-glycinate and iron bis-glycinate co-supplemented with vitamin C in women of reproductive age upon the same cognitive and behavioural outcomes assessed in Chapter 2. The findings provide novel evidence of the efficacy of a low-dose iron bis-glycinate chelate supplement for significantly improving haemoglobin when co-supplemented with vitamin C, and serum ferritin when administered alone or with vitamin C. However, no significant benefit of co-supplementation with vitamin C compared to iron alone was identified. The main effects of treatment were inconsistent; compared to placebo, iron alone significantly reduced PCS, indicating lower physical health, yet also significantly reduced ratings of depression-dejection and total mood disturbance. Furthermore, the iron and vitamin C treatment significantly reduced menstrual blood loss scores compared to placebo. However, in contrast to the Chapter 3 systematic review, no treatment effects were found for cognitive, fatigue or wellbeing outcomes indicating that a low dose of iron was perhaps not sufficient to elicit such changes in a general population sample of non-anaemic women of reproductive age. Nonetheless, the findings from Chapter 4 are the first concerning the effects of a low-dose iron bis-glycinate formula for improving iron status in NAID and IS women of reproductive age and has therefore demonstrated its ability to significantly improve iron status parameters compared to placebo with minimal associated adverse events.

The study presented in Chapter 5 is the first to investigate the parallel effects of iron supplementation on cerebral haemodynamics and energy metabolism in a sample of NAID and IS women of reproductive age. The study identified novel evidence of greater levels of total haemoglobin in the prefrontal cortex for NAID women during cognitive demand compared to IS women, which may act as a compensatory mechanism to enable the same standard of

cognitive performance across iron status groups. However, no significant effects upon cerebral haemodynamics or energy metabolism were found following the intervention period despite a significant improvement in the number of errors made on a task of working memory for the iron and vitamin C group. Considering this was the only cognitive finding through this thesis, future research should consider administering a working memory task battery of incremental difficulty for longer durations with longer rest periods between task repetitions to minimise crossover effects between the tasks. Further investigations with methodologies that are capable of monitoring brain activity and brain iron content, such as EEG, fMRI, multi-channel NIRS and QMS, should be considered simultaneously with ICa to elucidate a greater understanding of the mechanisms that underpin the effects discussed.

In summary, the findings of the thesis are suggestive of cognitive function not declining in the early stages of ID and provide novel insight into how this may be a consequence of increased compensatory CBF to ensure maintenance of cognitive and behavioural function in women of reproductive age. Iron bis-glycinate has shown positive evidence for improving iron status at low doses, however there is no significant benefit of vitamin C co-supplementation. Iron treatment alone should be considered beneficial for improving measures of depression and total mood disturbance, but research is required to investigate further the unexpected finding regarding reduced PCS. However, it is essential to reduce the noise associated with iron status classifications and to investigate the mechanisms that underpin the observed effects of iron on cognitive function, mood, fatigue, well-being, CBF and energy metabolism in women of reproductive age. Altogether, the findings have offered an intriguing insight into the role of iron for women's health that offer exciting opportunities for future research.

REFERENCES

Abbas, A. M., Abdelbadee, S. A., Alanwar, A., & Mostafa, S. (2019). Efficacy of ferrous bis-glycinate versus ferrous glycine sulfate in the treatment of iron deficiency anemia with pregnancy: a randomized double-blind clinical trial. *The Journal of Maternal-Fetal & Neonatal Medicine, 32*(24), 4139-4145. doi:10.1080/14767058.2018.1482871

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences, 19*(2), 164-174. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24778671

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3999603/

Abdel Moety, G. A. F., Ali, A. M., Fouad, R., Ramadan, W., Belal, D. S., & Haggag, H. M. (2017). Amino acid chelated iron versus an iron salt in the treatment of iron deficiency anemia with pregnancy: A randomized controlled study. *Eur J Obstet Gynecol Reprod Biol, 210*, 242-246. doi:10.1016/j.ejogrb.2017.01.003

Abu-Ouf, N. M., & Jan, M. M. (2015). The impact of maternal iron deficiency and iron deficiency anemia on child's health. *Saudi medical journal, 36*(2), 146-149. doi:10.15537/smj.2015.2.10289

Adane, A. A., Mishra, G. D., & Tooth, L. R. (2016). Diabetes in Pregnancy and Childhood Cognitive Development: A Systematic Review. *Pediatrics, 137*(5), e20154234. doi:10.1542/peds.2015-4234

Agarwal, K. N. (2001). Iron and the brain: neurotransmitter receptors and magnetic resonance spectroscopy. *Br J Nutr, 85 Suppl 2*, S147-150.

Agrawal, S., Berggren, K. L., Marks, E., & Fox, J. H. (2017). Impact of high iron intake on cognition and neurodegeneration in humans and in animal models: a systematic review. *Nutrition Reviews, 75*(6), 456-470. doi:10.1093/nutrit/nux015

Aigner, E., Feldman, A., & Datz, C. (2014). Obesity as an emerging risk factor for iron deficiency. *Nutrients, 6*(9), 3587-3600. doi:10.3390/nu6093587

Al-Naher, A., Schlaghecken, F., Barber, T., & Kumar, S. (2016). Modulation of metabolic rate in response to a simple cognitive task. *Arch Med, 8*(4), 1-7.

Albacar, G., Sans, T., Martin-Santos, R., Garcia-Esteve, L., Guillamat, R., Sanjuan, J., . . . Vilella, E. (2011). An association between plasma ferritin concentrations measured 48 h after delivery and postpartum depression. *J Affect Disord, 131*(1-3), 136-142. doi:10.1016/j.jad.2010.11.006

Algarin, C., Karunakaran, K. D., Reyes, S., Morales, C., Lozoff, B., Peirano, P., & Biswal, B. (2017). Differences on Brain Connectivity in Adulthood Are Present in Subjects with

Iron Deficiency Anemia in Infancy. *Frontiers in aging neuroscience, 9*(54). doi:10.3389/fnagi.2017.00054

Algarin, C., Nelson, C. A., Peirano, P., Westerlund, A., Reyes, S., & Lozoff, B. (2013). Iron-deficiency anemia in infancy and poorer cognitive inhibitory control at age 10 years. *Dev Med Child Neurol, 55*(5), 453-458. doi:10.1111/dmcn.12118

Algarín, C., Peirano, P., Garrido, M., Pizarro, F., & Lozoff, B. (2003). Iron Deficiency Anemia in Infancy: Long-Lasting Effects on Auditory and Visual System Functioning. *Pediatric Research, 53*(2), 217-223. doi:10.1203/01.PDR.0000047657.23156.55

Aliefendioglu, D., Yilmaz, S., Misirlioglu, E. D., Saygi, S., Ozdogan, S., & Kocak, U. (2007). Do cerebral blood flow velocities change in iron deficiency anemia? *J Pediatr Hematol Oncol, 29*(11), 747-751. doi:10.1097/MPH.0b013e318157fd85

Allen, E. A., Pasley, B. N., Duong, T., & Freeman, R. D. (2007). Transcranial Magnetic Stimulation Elicits Coupled Neural and Hemodynamic Consequences. *Science, 317*(5846), 1918-1921. doi:10.1126/science.1146426

Alleyne, M., Horne, M. K., & Miller, J. L. (2008). Individualized treatment for iron-deficiency anemia in adults. *The American journal of medicine, 121*(11), 943-948. doi:10.1016/j.amjmed.2008.07.012

Aly, S. S., Fayed, H. M., Ismail, A. M., & Abdel Hakeem, G. L. (2018). Assessment of peripheral blood lymphocyte subsets in children with iron deficiency anemia. *BMC Pediatrics, 18*(1), 49. doi:10.1186/s12887-018-0990-5

Ambrosini, G. L., Hurworth, M., Giglia, R., Trapp, G., & Strauss, P. (2018). Feasibility of a commercial smartphone application for dietary assessment in epidemiological research and comparison with 24-h dietary recalls. *Nutrition journal, 17*(1), 5. doi:10.1186/s12937-018-0315-4

Amiri, S., Behnezhad, S., & Nadinlui, K. B. (2018). Body Mass Index (BMI) and risk of depression in adults: A systematic review and meta-analysis of longitudinal studies. *Obesity Medicine, 12*, 1-12. doi:https://doi.org/10.1016/j.obmed.2018.10.001

Anderson, G. J., & Frazer, D. M. (2017). Current understanding of iron homeostasis. *The American journal of clinical nutrition, 106*(Suppl 6), 1559S-1566S. doi:10.3945/ajcn.117.155804

Angulo-Barroso, R. M., Li, M., Santos, D. C. C., Bian, Y., Sturza, J., Jiang, Y., . . . Lozoff, B. (2016). Iron Supplementation in Pregnancy or Infancy and Motor Development: A Randomized Controlled Trial. *Pediatrics, 137*(4), e20153547. doi:10.1542/peds.2015-3547

Anokye, R., Acheampong, E., Budu-Ainooson, A., Obeng, E. I., & Akwasi, A. G. (2018). Prevalence of postpartum depression and interventions utilized for its management. *Annals of general psychiatry, 17*, 18-18. doi:10.1186/s12991-018-0188-0

Ashmead, H. D. (2001). The absorption and metabolism of iron amino acid chelate. *Arch Latinoam Nutr, 51*(1 Suppl 1), 13-21.

Ashmead, S. D. (2001). The chemistry of ferrous bis-glycinate chelate. *Arch Latinoam Nutr, 51*(1 Suppl 1), 7-12.

Ashraf, A., Clark, M., & So, P.-W. (2018). The Aging of Iron Man. *Frontiers in aging neuroscience, 10*(65). doi:10.3389/fnagi.2018.00065

Aslan, M., Horoz, M., Kocyigit, A., Ozgonul, S., Celik, H., Celik, M., & Erel, O. (2006). Lymphocyte DNA damage and oxidative stress in patients with iron deficiency anemia. *Mutat Res, 601*(1-2), 144-149. doi:10.1016/j.mrfmmm.2006.06.013

Attia, M. A., Essa, S. A., Nosair, N. A., Amin, A. M., & El-Agamy, O. A. (2009). Effect of iron deficiency anemia and its treatment on cell mediated immunity. *Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion, 25*(2), 70-77. doi:10.1007/s12288-009-0017-3

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab, 21*(10), 1133-1145. doi:10.1097/00004647-200110000-00001

Augner, C. (2011). Associations of subjective sleep quality with depression score, anxiety, physical symptoms and sleep onset latency in students. *Cent Eur J Public Health, 19*(2), 115-117.

Badaracco, M. E., Siri, M. V. R., & Pasquini, J. M. (2010). Oligodendrogenesis: The role of iron. *BioFactors, 36*(2), 98-102. doi:10.1002/biof.90

Bagna, R., Spada, E., Mazzone, R., Saracco, P., Boetti, T., Cester, E. A., . . . Coscia, A. (2018). Efficacy of Supplementation with Iron Sulfate Compared to Iron Bisglycinate Chelate in Preterm Infants. *Current pediatric reviews, 14*(2), 123-129. doi:10.2174/1573396314666180124101059

Bahadir, A., Erduran, E., Değer, O., Birinci, Y., & Ayar, A. (2018). Augmented mitochondrial cytochrome c oxidase activity in children with iron deficiency: a tandem between iron and copper? *Archives of medical science : AMS, 14*(1), 151-156. doi:10.5114/aoms.2016.59602

Bahrami, A., Khorasanchi, Z., Tayefi, M., Avan, A., Seifi, N., Tavakoly Sany, S. B., . . . Ghayour-Mobarhan, M. (2019). Anemia is associated with cognitive impairment in adolescent girls: A cross-sectional survey. *Applied neuropsychology. Child*, 1-7. doi:10.1080/21622965.2018.1550405

Bailey, R. L., Fulgoni, V. L., 3rd, Keast, D. R., & Dwyer, J. T. (2012). Examination of vitamin intakes among US adults by dietary supplement use. *J Acad Nutr Diet, 112*(5), 657-663.e654. doi:10.1016/j.jand.2012.01.026

Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R., & Dwyer, J. T. (2013). Why US Adults Use Dietary SupplementsWhy US Adults Use Dietary Supplements. *JAMA Internal Medicine, 173*(5), 355-361. doi:10.1001/jamainternmed.2013.2299

Baines, S., Powers, J., & Brown, W. J. (2007). How does the health and well-being of young Australian vegetarian and semi-vegetarian women compare with non-vegetarians? *Public Health Nutr, 10*(5), 436-442. doi:10.1017/s1368980007217938

Baird-Gunning, J., & Bromley, J. (2016). Correcting iron deficiency. *Aust Prescr, 39*(6), 193-199. doi:10.18773/austprescr.2016.069

Bairwa, G., Hee Jung, W., & Kronstad, J. W. (2017). Iron acquisition in fungal pathogens of humans. *Metallomics : integrated biometal science, 9*(3), 215-227. doi:10.1039/c6mt00301j

Ballin, A., Berar, M., Rubinstein, U., Kleter, Y., Hershkovitz, A., & Meytes, D. (1992). Iron state in female adolescents. *Am J Dis Child, 146*(7), 803-805. doi:10.1001/archpedi.1992.02160190035015

Bani, S., Hassanpour-Siahestalkhi, A., Hassanpour, S., Mommad-Alizadeh-Charandabi, S., Mirghafourvand, M., & Javadzadeh, Y. (2014). Comparison of two iron supplementation methods on Hemoglobin level and Menstrual Bleeding in Tabriz students. *Iranian journal of pediatric hematology and oncology, 4*(1), 11-16. Retrieved from https://pubmed.ncbi.nlm.nih.gov/24734158

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3980016/

Barsky, A. J. (2017). The iatrogenic potential of the physician's words. *JAMA, 318*(24), 2425-2426.

Bartzokis, G., Lu, P. H., Tingus, K., Peters, D. G., Amar, C. P., Tishler, T. A., . . . Connor, J. R. (2011). Gender and iron genes may modify associations between brain iron and memory in healthy aging. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 36*(7), 1375-1384. doi:10.1038/npp.2011.22

Bastian, T. W., von Hohenberg, W. C., Georgieff, M. K., & Lanier, L. M. (2019). Chronic Energy Depletion due to Iron Deficiency Impairs Dendritic Mitochondrial Motility during Hippocampal Neuron Development. *The Journal of Neuroscience, 39*(5), 802. doi:10.1523/JNEUROSCI.1504-18.2018

Bastian, T. W., von Hohenberg, W. C., Mickelson, D. J., Lanier, L. M., & Georgieff, M. K. (2016). Iron Deficiency Impairs Developing Hippocampal Neuron Gene Expression, Energy Metabolism, and Dendrite Complexity. *Dev Neurosci, 38*(4), 264-276. doi:10.1159/000448514

Baumgartner, J., Smuts, C. M., Malan, L., Kvalsvig, J., van Stuijvenberg, M. E., Hurrell, R. F., & Zimmermann, M. B. (2012). Effects of iron and n-3 fatty acid supplementation,

alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa. *The American journal of clinical nutrition, 96*(6), 1327-1338. doi:10.3945/ajcn.112.041004

Baune, B. T., Fuhr, M., Air, T., & Hering, C. (2014). Neuropsychological functioning in adolescents and young adults with major depressive disorder--a review. *Psychiatry Res, 218*(3), 261-271. doi:10.1016/j.psychres.2014.04.052

Beard, J. (1995). One person's view of iron deficiency, development, and cognitive function. *The American journal of clinical nutrition, 62*(4), 709-710. doi:10.1093/ajcn/62.4.709

Beard, J. (2003). Iron deficiency alters brain development and functioning. *J Nutr, 133*(5 Suppl 1), 1468s-1472s. doi:10.1093/jn/133.5.1468S

Beard, J., & Tobin, B. (2000). Iron status and exercise. *The American journal of clinical nutrition, 72*(2), 594S-597S. doi:10.1093/ajcn/72.2.594S

Beard, J. L. (2000). Iron requirements in adolescent females. *The Journal of nutrition, 130*(2S Suppl), 440S-442S. doi:10.1093/jn/130.2.440S

Beard, J. L. (2001). Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. *The Journal of nutrition, 131*(2), 568S-580S. doi:10.1093/jn/131.2.568S

Beard, J. L., & Connor, J. R. (2003). Iron status and neural functioning. *Annu Rev Nutr, 23*, 41-58. doi:10.1146/annurev.nutr.23.020102.075739

Beard, J. L., Erikson, K. M., & Jones, B. C. (2002). Neurobehavioral analysis of developmental iron deficiency in rats. *Behav Brain Res, 134*(1-2), 517-524. doi:10.1016/s0166-4328(02)00092-x

Beard, J. L., Felt, B., Schallert, T., Burhans, M., Connor, J. R., & Georgieff, M. K. (2006). Moderate iron deficiency in infancy: biology and behavior in young rats. *Behav Brain Res, 170*(2), 224-232. doi:10.1016/j.bbr.2006.02.024

Beard, J. L., Hendricks, M. K., Perez, E. M., Murray-Kolb, L. E., Berg, A., Vernon-Feagans, L., . . . Tomlinson, M. (2005). Maternal Iron Deficiency Anemia Affects Postpartum Emotions and Cognition. *The Journal of nutrition, 135*(2), 267-272. doi:10.1093/jn/135.2.267

Beard, J. L., Wiesinger, J. A., & Connor, J. R. (2003). Pre- and postweaning iron deficiency alters myelination in Sprague-Dawley rats. *Dev Neurosci, 25*(5), 308-315. doi:10.1159/000073507

Beck, K., Conlon, C. A., Kruger, R., Coad, J., & Stonehouse, W. (2011). Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. *British Journal of Nutrition, 105*(1), 101-109. doi:10.1017/S0007114510003144

Beck, K. L., Conlon, C. A., Kruger, R., & Coad, J. (2014). Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: a review. *Nutrients, 6*(9), 3747-3776. doi:10.3390/nu6093747

Beck, K. L., Conlon, C. A., Kruger, R., Heath, A.-L. M., Matthys, C., Coad, J., & Stonehouse, W. (2012). Iron Status and Self-Perceived Health, Well-Being, and Fatigue in Female University Students Living in New Zealand. *J Am Coll Nutr, 31*(1), 45-53. doi:10.1080/07315724.2012.10720008

Beltran-Navarro, B., Matute, E., Vasquez-Garibay, E., & Zarabozo, D. (2012). Effect of chronic iron deficiency on neuropsychological domains in infants. *J Child Neurol, 27*(3), 297-303. doi:10.1177/0883073811416867

Benton, M. J., Hutchins, A. M., & Dawes, J. J. (2020). Effect of menstrual cycle on resting metabolism: A systematic review and meta-analysis. *PloS one, 15*(7), e0236025-e0236025. doi:10.1371/journal.pone.0236025

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry, Fifth Edition*: W.H. Freeman.

Berger, T., Togawa, A., Duncan, G. S., Elia, A. J., You-Ten, A., Wakeham, A., . . . Mak, T. W. (2006). Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. *Proc Natl Acad Sci U S A, 103*(6), 1834-1839. doi:10.1073/pnas.0510847103

Berggren, K. L., Lu, Z., Fox, J. A., Dudenhoeffer, M., Agrawal, S., & Fox, J. H. (2016). Neonatal Iron Supplementation Induces Striatal Atrophy in Female YAC128 Huntington's Disease Mice. *Journal of Huntington's Disease, 5*(1), 53-63. doi:10.3233/JHD-150182

Berglund, S. K., Chmielewska, A., Starnberg, J., Westrup, B., Hagglof, B., Norman, M., & Domellof, M. (2018). Effects of iron supplementation of low-birth-weight infants on cognition and behavior at 7 years: a randomized controlled trial. *Pediatr Res, 83*(1-1), 111-118. doi:10.1038/pr.2017.235

Berglund, S. K., Westrup, B., Hagglof, B., Hernell, O., & Domellof, M. (2013). Effects of iron supplementation of LBW infants on cognition and behavior at 3 years. *Pediatrics, 131*(1), 47-55. doi:10.1542/peds.2012-0989

Bertsch, K., Hagemann, D., Hermes, M., Walter, C., Khan, R., & Naumann, E. (2009). Resting cerebral blood flow, attention, and aging. *Brain Res, 1267*, 77-88. doi:10.1016/j.brainres.2009.02.053

Beutler, E., Larsh, S. E., & Gurney, C. W. (1960). IRON THERAPY IN CHRONICALLY FATIGUED, NONANEMIC WOMEN: A DOUBLE-BLIND STUDY*. *Annals of Internal Medicine, 52*(2), 378-394. doi:10.7326/0003-4819-52-2-378

Bhavi, S. B., & Jaju, P. B. (2017). Intravenous iron sucrose v/s oral ferrous fumarate for treatment of anemia in pregnancy. A randomized controlled trial. *BMC pregnancy and childbirth, 17*(1), 137-137. doi:10.1186/s12884-017-1313-9

Biagioli, M., Pinto, M., Cesselli, D., Zaninello, M., Lazarevic, D., Roncaglia, P., . . . Gustincich, S. (2009). Unexpected expression of alpha- and beta-globin in mesencephalic dopaminergic neurons and glial cells. *Proc Natl Acad Sci U S A, 106*(36), 15454-15459. doi:10.1073/pnas.0813216106

Bianco, L. E., Wiesinger, J., Earley, C. J., Jones, B. C., & Beard, J. L. (2008). Iron deficiency alters dopamine uptake and response to L-DOPA injection in Sprague-Dawley rats. *J Neurochem, 106*(1), 205-215. doi:10.1111/j.1471-4159.2008.05358.x

Binkoski, A. E., Kris-Etherton, P. M., & Beard, J. L. (2004). Iron supplementation does not affect the susceptibility of LDL to oxidative modification in women with low iron status. *The Journal of nutrition, 134*(1), 99-103.

Blanco-Rojo, R., Baeza-Richer, C., López-Parra, A. M., Pérez-Granados, A. M., Brichs, A., Bertoncini, S., . . . Vaquero, M. P. (2011). Four variants in transferrin and HFE genes as potential markers of iron deficiency anaemia risk: an association study in menstruating women. *Nutrition & Metabolism, 8*, 69-69. doi:10.1186/1743-7075-8-69

Blanco-Rojo, R., Maria Perez-Granados, A., Lopez-Parra, A. M., Baeza, C., Bertoncini, S., Arroyo-Pardo, E., & Vaquero, P. (2010). *Variants in transferrin gene affect iron metabolism and response to an iron supplemented food in menstruating women.* Paper presented at the Journal of Nutrigenetics and Nutrigenomics.

Blanco-Rojo, R., Toxqui, L., López-Parra, A. M., Baeza-Richer, C., Pérez-Granados, A. M., Arroyo-Pardo, E., & Vaquero, M. P. (2014). Influence of diet, menstruation and genetic factors on iron status: a cross-sectional study in Spanish women of childbearing age. *International journal of molecular sciences, 15*(3), 4077-4087. doi:10.3390/ijms15034077

Blanton, C. (2013). Improvements in iron status and cognitive function in young women consuming beef or non-beef lunches. *Nutrients, 6*(1), 90-110. doi:10.3390/nu6010090

Blanton, C. A., Green, M. W., & Kretsch, M. J. (2013). Body iron is associated with cognitive executive planning function in college women. *Br J Nutr, 109*(5), 906-913. doi:10.1017/s0007114512002620

Bloomfield, M. A., McCutcheon, R. A., Kempton, M., Freeman, T. P., & Howes, O. (2019). The effects of psychosocial stress on dopaminergic function and the acute stress response. *Elife, 8*. doi:10.7554/eLife.46797

Bluhm, R., Williamson, P., Lanius, R., Theberge, J., Densmore, M., Bartha, R., . . . Osuch, E. (2009). Resting state default-mode network connectivity in early depression using

a seed region-of-interest analysis: decreased connectivity with caudate nucleus. *Psychiatry Clin Neurosci, 63*(6), 754-761. doi:10.1111/j.1440-1819.2009.02030.x

Blumberg, J. B., Bailey, R. L., Sesso, H. D., & Ulrich, C. M. (2018). The Evolving Role of Multivitamin/Multimineral Supplement Use among Adults in the Age of Personalized Nutrition. *Nutrients, 10*(2). doi:10.3390/nu10020248

Bodnar, L. M., & Wisner, K. L. (2005). Nutrition and depression: implications for improving mental health among childbearing-aged women. *Biological Psychiatry, 58*(9), 679-685. doi:10.1016/j.biopsych.2005.05.009

Bogan, R. K. (2006). Effects of restless legs syndrome (RLS) on sleep. *Neuropsychiatric disease and treatment, 2*(4), 513-519. doi:10.2147/nedt.2006.2.4.513

Bond, M.M., & Richards-Kortum, R.R. (2015). Drop-to-Drop Variation in the Cellular Components of Fingerprick Blood: Implications for Point-of-Care Diagnostic Development. *Am J Clin Pathol, 144*, 885–894

Borel, M. J., Smith, S. M., Derr, J., & Beard, J. L. (1991). Day-to-day variation in iron-status indices in healthy men and women. *The American journal of clinical nutrition, 54*(4), 729-735.

Bothwell, T. H. (2000). Iron requirements in pregnancy and strategies to meet them. *The American journal of clinical nutrition, 72*(1 Suppl), 257S-264S. doi:10.1093/ajcn/72.1.257S

Bovell-Benjamin, A. C., Viteri, F. E., & Allen, L. H. (2000). Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *The American journal of clinical nutrition, 71*(6), 1563-1569. doi:10.1093/ajcn/71.6.1563

Brady, S., Siegel, G., Albers, R. W., & Price, D. (2005). *Basic neurochemistry: molecular, cellular and medical aspects*: Elsevier.

Brannon, P. M., & Taylor, C. L. (2017). Iron Supplementation during Pregnancy and Infancy: Uncertainties and Implications for Research and Policy. *Nutrients, 9*(12). doi:10.3390/nu9121327

Broberg, C. S., Bax, B. E., Okonko, D. O., Rampling, M. W., Bayne, S., Harries, C., . . . Gatzoulis, M. A. (2006). Blood viscosity and its relationship to iron deficiency, symptoms, and exercise capacity in adults with cyanotic congenital heart disease. *J Am Coll Cardiol, 48*(2), 356-365. doi:10.1016/j.jacc.2006.03.040

Brownlie IV, T., Utermohlen, V., Hinton, P. S., Giordano, C., & Haas, J. D. (2002). Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. *The American journal of clinical nutrition, 75*(4), 734-742.

Bruner, A. B., Joffe, A., Duggan, A. K., Casella, J. F., & Brandt, J. (1996). Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet, 348*(9033), 992-996. doi:10.1016/s0140-6736(96)02341-0

Brunette, K. E., Tran, P. V., Wobken, J. D., Carlson, E. S., & Georgieff, M. K. (2010). Gestational and neonatal iron deficiency alters apical dendrite structure of CA1 pyramidal neurons in adult rat hippocampus. *Dev Neurosci, 32*(3), 238-248. doi:10.1159/000314341

Brutsaert, T. D., Hernandez-Cordero, S., Rivera, J., Viola, T., Hughes, G., & Haas, J. D. (2003). Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. *The American journal of clinical nutrition, 77*(2), 441-448.

Brynskikh, A., Warren, T., Zhu, J., & Kipnis, J. (2008). Adaptive immunity affects learning behavior in mice. *Brain Behav Immun, 22*(6), 861-869. doi:10.1016/j.bbi.2007.12.008

Buratti, P., Gammella, E., Rybinska, I., Cairo, G., & Recalcati, S. (2015). Recent Advances in Iron Metabolism: Relevance for Health, Exercise, and Performance. *Med Sci Sports Exerc, 47*(8), 1596-1604. doi:10.1249/mss.0000000000000593

Burden, R. J., Morton, K., Richards, T., Whyte, G. P., & Pedlar, C. R. (2015). Is iron treatment beneficial in, iron-deficient but non-anaemic (IDNA) endurance athletes? A systematic review and meta-analysis. *Br J Sports Med, 49*(21), 1389-1397. doi:10.1136/bjsports-2014-093624

Burden, R. J., Pollock, N., Whyte, G. P., Richards, T., Moore, B., Busbridge, M., . . . Pedlar, C. R. (2015). Effect of Intravenous Iron on Aerobic Capacity and Iron Metabolism in Elite Athletes. *Med Sci Sports Exerc, 47*(7), 1399-1407. doi:10.1249/mss.0000000000000568

Caballero, B., Trugo, L. C., & Finglas, P. M. (2003). *Encyclopedia of food sciences and nutrition*: Academic.

Cable, R. G., Brambilla, D., Glynn, S. A., Kleinman, S., Mast, A. E., Spencer, B. R., . . . Donor Evaluation, S., III. (2016). Effect of iron supplementation on iron stores and total body iron after whole blood donation. *Transfusion, 56*(8), 2005-2012. doi:10.1111/trf.13659

Cammer, W. (1984). Oligodendrocyte-associated enzymes. In *Oligodendroglia* (pp. 199-232): Springer.

Cammer, W., Snyder, D. S., Zimmerman, T. R., Jr., Farooq, M., & Norton, W. T. (1982). Glycerol phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase: activities in oligodendrocytes, neurons, astrocytes, and myelin isolated from developing rat brains. *J Neurochem, 38*(2), 360-367. doi:10.1111/j.1471-4159.1982.tb08637.x

Campbell, C., Mallappa, A., Wisniewski, A. B., & Silovsky, J. F. (2013). Chapter 6 - Sexual Behavior of Prepubertal Children. In D. S. Bromberg & W. T. O'Donohue (Eds.),

Handbook of Child and Adolescent Sexuality (pp. 145-170). San Diego: Academic Press.

Camprubi Robles, M., Campoy, C., Garcia Fernandez, L., Lopez-Pedrosa, J. M., Rueda, R., & Martin, M. J. (2015). Maternal Diabetes and Cognitive Performance in the Offspring: A Systematic Review and Meta-Analysis. *PLoS One, 10*(11), e0142583-e0142583. doi:10.1371/journal.pone.0142583

Campus, P., Canterini, S., Orsini, C., Fiorenza, M. T., Puglisi-Allegra, S., & Cabib, S. (2017). Stress-induced reduction of dorsal striatal D2 dopamine receptors prevents retention of a newly acquired adaptive coping strategy. *Front Pharmacol, 8*, 621.

Cantarero-Villanueva, I., Fernandez-Lao, C., Diaz-Rodriguez, L., Cuesta-Vargas, A. I., Fernandez-de-las-Penas, C., Piper, B. F., & Arroyo-Morales, M. (2014). The Piper Fatigue Scale-Revised: translation and psychometric evaluation in Spanish-speaking breast cancer survivors. *Qual Life Res, 23*(1), 271-276. doi:10.1007/s11136-013-0434-5

Carlson, E. S., Stead, J. D., Neal, C. R., Petryk, A., & Georgieff, M. K. (2007). Perinatal iron deficiency results in altered developmental expression of genes mediating energy metabolism and neuronal morphogenesis in hippocampus. *Hippocampus, 17*(8), 679-691. doi:10.1002/hipo.20307

Carr, A. C., Bozonet, S. M., Pullar, J. M., & Vissers, M. C. (2013). Mood improvement in young adult males following supplementation with gold kiwifruit, a high-vitamin C food. *J Nutr Sci, 2*, e24. doi:10.1017/jns.2013.12

Carrier, J., Aghdassi, E., Cullen, J., & Allard, J. P. (2002). Iron supplementation increases disease activity and vitamin E ameliorates the effect in rats with dextran sulfate sodium-induced colitis. *The Journal of nutrition, 132*(10), 3146-3150.

Carter, R. C., Jacobson, J. L., Burden, M. J., Armony-Sivan, R., Dodge, N. C., Angelilli, M. L., . . . Jacobson, S. W. (2010). Iron deficiency anemia and cognitive function in infancy. *Pediatrics, 126*(2), e427-434. doi:10.1542/peds.2009-2097

Cashman, K. D., & Hayes, A. (2017). Red meat's role in addressing 'nutrients of public health concern'. *Meat Sci, 132*, 196-203. doi:10.1016/j.meatsci.2017.04.011

Castellanos, F. X., Margulies, D. S., Kelly, C., Uddin, L. Q., Ghaffari, M., Kirsch, A., . . . Milham, M. P. (2008). Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry, 63*(3), 332-337. doi:10.1016/j.biopsych.2007.06.025

Catchlove, S. J., Macpherson, H., Hughes, M. E., Chen, Y., Parrish, T. B., & Pipingas, A. (2018). An investigation of cerebral oxygen utilization, blood flow and cognition in healthy aging. *PLoS One, 13*(5), e0197055. doi:10.1371/journal.pone.0197055

Charlson, F., van Ommeren, M., Flaxman, A., Cornett, J., Whiteford, H., & Saxena, S. (2019). New WHO prevalence estimates of mental disorders in conflict settings: a systematic review and meta-analysis. *The Lancet, 394*(10194), 240-248. doi:https://doi.org/10.1016/S0140-6736(19)30934-1

Chełchowska, M., Laskowska-Klita, T., & Leibschang, J. (2007). [Concentration of ferritin, transferrin and iron as a markers of iron deficiency in healthy women in reproductive age]. *Pol Merkur Lekarski, 22*(127), 25-27.

Chen, M.-H., Su, T.-P., Chen, Y.-S., Hsu, J.-W., Huang, K.-L., Chang, W.-H., . . . Bai, Y.-M. (2013). Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. *BMC Psychiatry, 13*(1), 161. doi:10.1186/1471-244X-13-161

Chester, D. S., DeWall, C. N., Derefinko, K. J., Estus, S., Lynam, D. R., Peters, J. R., & Jiang, Y. (2016). Looking for reward in all the wrong places: dopamine receptor gene polymorphisms indirectly affect aggression through sensation-seeking. *Social neuroscience, 11*(5), 487-494. doi:10.1080/17470919.2015.1119191

Chhatwal, J. P., Schultz, A. P., Johnson, K., Benzinger, T. L., Jack, C., Jr., Ances, B. M., . . . Sperling, R. A. (2013). Impaired default network functional connectivity in autosomal dominant Alzheimer disease. *Neurology, 81*(8), 736-744. doi:10.1212/WNL.0b013e3182a1aafe

Chiabrando, D., Vinchi, F., Fiorito, V., Mercurio, S., & Tolosano, E. (2014). Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. *Front Pharmacol, 5*, 61. doi:10.3389/fphar.2014.00061

Chiamchanya, N. (2013). Rapid recovery time of hemoglobin level in female regular blood donors with ferrous fumarate and high dose of ascorbic acid supplement. *J Med Assoc Thai, 96*(2), 165-171.

Chiarelli, A. M., Zappasodi, F., Di Pompeo, F., & Merla, A. (2017). Simultaneous functional near-infrared spectroscopy and electroencephalography for monitoring of human brain activity and oxygenation: a review. *Neurophotonics, 4*(4), 041411-041411. doi:10.1117/1.NPh.4.4.041411

Chmielewska, A., Dziechciarz, P., Gieruszczak-Białek, D., Horvath, A., Pieścik-Lech, M., Ruszczyński, M., . . . Szajewska, H. (2019). Effects of prenatal and/or postnatal supplementation with iron, PUFA or folic acid on neurodevelopment: update. *British Journal of Nutrition, 122*(s1), S10-S15. doi:10.1017/S0007114514004243

Cho, G. J., Shin, J.-H., Yi, K. W., Park, H. T., Kim, T., Hur, J. Y., & Kim, S. H. (2011). Serum ferritin levels are associated with metabolic syndrome in postmenopausal women but not in premenopausal women. *Menopause (New York, N.Y.), 18*(10), 1120-1124. doi:10.1097/gme.0b013e318217e172

Choi, J., Pai, S., Kim, S., Ito, M., Park, C., & Cha, Y. (2002). Iron deficiency anemia increases nitric oxide production in healthy adolescents. *Annals of Hematology, 81*(1), 1-6. doi:10.1007/s00277-001-0409-4

Clark, P. C., Ashford, S., Burt, R., Aycock, D. M., & Kimble, L. P. (2006). Factor analysis of the Revised Piper Fatigue Scale in a caregiver sample. *J Nurs Meas, 14*(2), 71-78.

Cohen, J. F. W., Gorski, M. T., Gruber, S. A., Kurdziel, L. B. F., & Rimm, E. B. (2016). The effect of healthy dietary consumption on executive cognitive functioning in children and adolescents: a systematic review. *British Journal of Nutrition, 116*(6), 989-1000. doi:10.1017/S0007114516002877

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385-396. doi:10.2307/2136404

Collins, H. L. (2003). The role of iron in infections with intracellular bacteria. *Immunol Lett, 85*(2), 193-195. doi:10.1016/s0165-2478(02)00229-8

Connor, J. R., & Menzies, S. L. (1996). Relationship of iron to oligodendrocytes and myelination. *Glia, 17*(2), 83-93. doi:10.1002/(sici)1098-1136(199606)17:2<83::Aid-glia1>3.0.Co;2-7

Conrad, M. E., & Schade, S. G. (1968). Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile. *Gastroenterology, 55*(1), 35-45.

Constante, M., Fragoso, G., Lupien-Meilleur, J., Calvé, A., & Santos, M. M. (2017). Iron Supplements Modulate Colon Microbiota Composition and Potentiate the Protective Effects of Probiotics in Dextran Sodium Sulfate-induced Colitis. *Inflammatory Bowel Diseases, 23*(5), 753-766. doi:10.1097/mib.0000000000001089

Cook, J. D. (2005). Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol, 18*(2), 319-332. doi:10.1016/j.beha.2004.08.022

Cook, J. D., Boy, E., Flowers, C., & Daroca Mdel, C. (2005). The influence of high-altitude living on body iron. *Blood, 106*(4), 1441-1446. doi:10.1182/blood-2004-12-4782

Cook, J. D., Flowers, C. H., & Skikne, B. S. (2003). The quantitative assessment of body iron. *Blood, 101*(9), 3359-3364. doi:10.1182/blood-2002-10-3071

Cook, J. D., Skikne, B. S., Lynch, S. R., & Reusser, M. E. (1986). Estimates of iron sufficiency in the US population. *Blood, 68*(3), 726-731.

Cook, R. L., O'Dwyer, N. J., Donges, C. E., Parker, H. M., Cheng, H. L., Steinbeck, K. S., . . . O'Connor, H. T. (2017). Relationship between Obesity and Cognitive Function in Young Women: The Food, Mood and Mind Study. *J Obes, 2017*, 5923862. doi:10.1155/2017/5923862

Cook, R. L., O'Dwyer, N. J., Parker, H. M., Donges, C. E., Cheng, H. L., Steinbeck, K. S., . . . O'Connor, H. T. (2017). Iron Deficiency Anemia, Not Iron Deficiency, Is Associated

with Reduced Attention in Healthy Young Women. *Nutrients, 9*(11), 1216. doi:10.3390/nu9111216

Copher, R., Nestour, E., Zampaglione, E., Prezioso, A. N., Pocoski, J., & Law, A. (2012). Heavy menstrual bleeding treatment patterns and associated health care utilization and costs. *Journal of Clinical Outcomes Management, 19*, 402-413.

Coplin, M., Schuette, S., Leichtmann, G., & Lashner, B. (1991). Tolerability of iron: a comparison of bis-glycino iron II and ferrous sulfate. *Clin Ther, 13*(5), 606-612.

Corey, S. J., Kimmel, M., & Leonard, J. N. (2014). *A Systems Biology Approach to Blood* (Vol. 844): Springer.

Cornelli, U., & Belcaro, G. (2015). Treatment of Anemia Owing to Increased Menstrual Blood Loss: Activity of Physiological Modulators. *Journal of Hematology, 4*, 164-170. doi:10.14740/jh215w

Corwin, E. J., Murray-Kolb, L. E., & Beard, J. L. (2003). Low hemoglobin level is a risk factor for postpartum depression. *J Nutr, 133*(12), 4139-4142. doi:10.1093/jn/133.12.4139

Coşkun, Ş., Bilgen, H., Özdemir, H., Şirikçi, Ö., & Özek, E. (2012). 751 Are Infants of Diabetic Mothers More Prone to Iron Deficiency? *Archives of Disease in Childhood, 97*(Suppl 2), A216-A216. doi:10.1136/archdischild-2012-302724.0751

Covarrubias-Pinto, A., Acuna, A. I., Beltran, F. A., Torres-Diaz, L., & Castro, M. A. (2015). Old Things New View: Ascorbic Acid Protects the Brain in Neurodegenerative Disorders. *International journal of molecular sciences, 16*(12), 28194-28217. doi:10.3390/ijms161226095

Cox, E. P., O'Dwyer, N., Cook, R., Vetter, M., Cheng, H. L., Rooney, K., & O'Connor, H. (2016). Relationship between physical activity and cognitive function in apparently healthy young to middle-aged adults: A systematic review. *J Sci Med Sport, 19*(8), 616-628. doi:10.1016/j.jsams.2015.09.003

Craig, C. L., Marshall, A. L., Sjostrom, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., . . . Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc, 35*(8), 1381-1395. doi:10.1249/01.Mss.0000078924.61453.Fb

Crouter, S. E., DellaValle, D. M., & Haas, J. D. (2012). Relationship between physical activity, physical performance, and iron status in adult women. *Appl Physiol Nutr Metab, 37*(4), 697-705. doi:10.1139/h2012-044

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., . . . Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiological Reviews, 99*(4), 1877-2013. doi:10.1152/physrev.00018.2018

Cui, X., Bray, S., Bryant, D. M., Glover, G. H., & Reiss, A. L. (2011). A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. *NeuroImage, 54*(4), 2808-2821. doi:10.1016/j.neuroimage.2010.10.069

Cullen, W., Kearney, Y., & Bury, G. (2002). Prevalence of fatigue in general practice. *Irish Journal of Medical Science, 171*(1), 10. doi:10.1007/BF03168931

Curran, S., Andrykowski, M., & Studts, J. (1995). Short form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment, 7*, 80-83. doi:10.1037/1040-3590.7.1.80

da Silva Pereira, A., de Castro, I., Bezerra F.F., Nogueira Neto, J.F., & da Silva, A. (2020). Reproducibility and validity of portable haemoglobinometer for the diagnosis of anaemia in children under the age of 5 years. *J Nutr Sci*, *9*, e3

D'Adamo, C. R., Novick, J. S., Feinberg, T. M., Dawson, V. J., & Miller, L. E. (2018). A Food-Derived Dietary Supplement Containing a Low Dose of Iron Improved Markers of Iron Status Among Nonanemic Iron-Deficient Women. *J Am Coll Nutr, 37*(4), 342-349. doi:10.1080/07315724.2018.1427158

D'Angelo, G. (2013). Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Res, 48*(1), 10-15. doi:10.5045/br.2013.48.1.10

Dajnowicz, S., Seaver, S., Hanson, B. L., Fisher, S. Z., Langan, P., Kovalevsky, A. Y., & Mueser, T. C. (2016). Visualizing the Bohr effect in hemoglobin: neutron structure of equine cyanomethemoglobin in the R state and comparison with human deoxyhemoglobin in the T state. *Acta crystallographica. Section D, Structural biology, 72*(Pt 7), 892-903. doi:10.1107/S2059798316009049

Dallman, P. R. (1986). Biochemical basis for the manifestations of iron deficiency. *Annu Rev Nutr, 6*, 13-40. doi:10.1146/annurev.nu.06.070186.000305

Daru, J., Colman, K., Stanworth, S. J., De La Salle, B., Wood, E. M., & Pasricha, S.-R. (2017). Serum ferritin as an indicator of iron status: what do we need to know? *The American journal of clinical nutrition, 106*(suppl_6), 1634S-1639S. doi:10.3945/ajcn.117.155960

Das, N. K., Schwartz, A. J., Barthel, G., Inohara, N., Liu, Q., Sankar, A., . . . Shah, Y. M. (2020). Microbial Metabolite Signaling Is Required for Systemic Iron Homeostasis. *Cell Metab, 31*(1), 115-130.e116. doi:10.1016/j.cmet.2019.10.005

Daugherty, A. M., Haacke, E. M., & Raz, N. (2015). Striatal iron content predicts its shrinkage and changes in verbal working memory after two years in healthy adults. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 35*(17), 6731-6743. doi:10.1523/JNEUROSCI.4717-14.2015

Daugherty, A. M., & Raz, N. (2016). Accumulation of iron in the putamen predicts its shrinkage in healthy older adults: A multi-occasion longitudinal study. *NeuroImage, 128*, 11-20. doi:10.1016/j.neuroimage.2015.12.045

Davies, D. J., Clancy, M., Lighter, D., Balanos, G. M., Lucas, S. J. E., Dehghani, H., . . . Belli, A. (2017). Frequency-domain vs continuous-wave near-infrared spectroscopy devices: a comparison of clinically viable monitors in controlled hypoxia. *J Clin Monit Comput, 31*(5), 967-974. doi:10.1007/s10877-016-9942-5

Davis, J. M., & Bailey, S. P. (1997). Possible mechanisms of central nervous system fatigue during exercise. *Medicine & Science in Sports & Exercise, 29*(1), 45-57.

Davis, K. L., Stewart, D. G., Friedman, J. I., Buchsbaum, M., Harvey, P. D., Hof, P. R., . . . Haroutunian, V. (2003). White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry, 60*(5), 443-456. doi:10.1001/archpsyc.60.5.443

de Back, D. Z., Kostova, E. B., van Kraaij, M., van den Berg, T. K., & van Bruggen, R. (2014). Of macrophages and red blood cells; a complex love story. *Front Physiol, 5*, 9. doi:10.3389/fphys.2014.00009

de Deungria, M., Rao, R., Wobken, J. D., Luciana, M., Nelson, C. A., & Georgieff, M. K. (2000). Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res, 48*(2), 169-176. doi:10.1203/00006450-200008000-00009

de Dreu, M. J., Schouwenaars, I. T., Rutten, G.-J. M., Ramsey, N. F., & Jansma, J. M. (2019). Brain Activity Associated With Expected Task Difficulty. *Frontiers in human neuroscience, 13*(286). doi:10.3389/fnhum.2019.00286

de Moura, J. E., de Moura, E. N., Alves, C. X., Vale, S. H., Dantas, M. M., Silva Ade, A., . . . Brandao-Neto, J. (2013). Oral zinc supplementation may improve cognitive function in schoolchildren. *Biol Trace Elem Res, 155*(1), 23-28. doi:10.1007/s12011-013-9766-9

de Oliveira, I. J., de Souza, V. V., Motta, V., & Da-Silva, S. L. (2015). Effects of Oral Vitamin C Supplementation on Anxiety in Students: A Double-Blind, Randomized, Placebo-Controlled Trial. *Pak J Biol Sci, 18*(1), 11-18. doi:10.3923/pjbs.2015.11.18

de Vivo, L., & Bellesi, M. (2019). The role of sleep and wakefulness in myelin plasticity. *Glia, 67*(11), 2142-2152. doi:10.1002/glia.23667

de Wit, A. E., Booij, S. H., Giltay, E. J., Joffe, H., Schoevers, R. A., & Oldehinkel, A. J. (2020). Association of Use of Oral Contraceptives With Depressive Symptoms Among Adolescents and Young Women. *JAMA Psychiatry, 77*(1), 52-59. doi:10.1001/jamapsychiatry.2019.2838

de Wit, L., Luppino, F., van Straten, A., Penninx, B., Zitman, F., & Cuijpers, P. (2010). Depression and obesity: a meta-analysis of community-based studies. *Psychiatry Res, 178*(2), 230-235. doi:10.1016/j.psychres.2009.04.015

Debener, S., Emkes, R., De Vos, M., & Bleichner, M. (2015). Unobtrusive ambulatory EEG using a smartphone and flexible printed electrodes around the ear. *Scientific reports, 5*(1), 16743. doi:10.1038/srep16743

Dehghan, M., Akhtar-Danesh, N., McMillan, C. R., & Thabane, L. (2007). Is plasma vitamin C an appropriate biomarker of vitamin C intake? A systematic review and meta-analysis. *Nutrition journal, 6*, 41-41. doi:10.1186/1475-2891-6-41

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *J Clin Psychiatry, 61 Suppl 6*, 7-11.

DellaValle, D. M. (2013). Iron supplementation for female athletes: effects on iron status and performance outcomes. *Curr Sports Med Rep, 12*(4), 234-239. doi:10.1249/JSR.0b013e31829a6f6b

DellaValle, D. M., & Haas, J. D. (2011). Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. *Int J Sport Nutr Exerc Metab, 21*(6), 501-506. doi:10.1123/ijsnem.21.6.501

Dellavalle, D. M., & Haas, J. D. (2014). Iron Supplementation Improves Energetic Efficiency in Iron-Depleted Female Rowers. *Medicine & Science in Sports & Exercise, 46*(6). Retrieved from https://journals.lww.com/acsm-msse/Fulltext/2014/06000/Iron_Supplementation_Improves_Energetic_Efficiency.17.aspx

DelRosso, L. M., Yi, T., Chan, J. H. M., Wrede, J. E., Lockhart, C. T., & Ferri, R. (2019). Determinants of ferritin response to oral iron supplementation in children with sleep movement disorders. *Sleep*. doi:10.1093/sleep/zsz234

Denny, S. D., Kuchibhatla, M. N., & Cohen, H. J. (2006). Impact of Anemia on Mortality, Cognition, and Function in Community-Dwelling Elderly. *The American journal of medicine, 119*(4), 327-334. doi:https://doi.org/10.1016/j.amjmed.2005.08.027

Deregnier, R. A., Nelson, C. A., Thomas, K. M., Wewerka, S., & Georgieff, M. K. (2000). Neurophysiologic evaluation of auditory recognition memory in healthy newborn infants and infants of diabetic mothers. *J Pediatr, 137*(6), 777-784. doi:10.1067/mpd.2000.109149

Devaki, P. B., Chandra, R. K., & Geisser, P. (2009). Effects of oral iron(III) hydroxide polymaltose complex supplementation on hemoglobin increase, cognitive function, affective behavior and scholastic performance of adolescents with varying iron

status: a single centre prospective placebo controlled study. *Arzneimittelforschung, 59*(6), 303-310. doi:10.1055/s-0031-1296401

Dewey, K. G., & Oaks, B. M. (2017). U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation. *The American journal of clinical nutrition, 106*(Suppl 6), 1694S-1702S. doi:10.3945/ajcn.117.156075

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., . . . Yang, W. S. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell, 149*(5), 1060-1072.

Dodd, F. L., Kennedy, D. O., Stevenson, E. J., Veasey, R. C., Walker, K., Reed, S., . . . Haskell-Ramsay, C. F. (2020). Acute and chronic effects of multivitamin/mineral supplementation on objective and subjective energy measures. *Nutrition & Metabolism, 17*(1), 16. doi:10.1186/s12986-020-00435-1

Dodell-Feder, D., Delisi, L. E., & Hooker, C. I. (2014). The relationship between default mode network connectivity and social functioning in individuals at familial high-risk for schizophrenia. *Schizophr Res, 156*(1), 87-95. doi:10.1016/j.schres.2014.03.031

Domínguez, R., Sánchez-Oliver, A. J., Mata-Ordoñez, F., Feria-Madueño, A., Grimaldi-Puyana, M., López-Samanes, Á., & Pérez-López, A. (2018). Effects of an Acute Exercise Bout on Serum Hepcidin Levels. *Nutrients, 10*(2), 209. doi:10.3390/nu10020209

Dosman, C. F., Brian, J. A., Drmic, I. E., Senthilselvan, A., Harford, M. M., Smith, R. W., . . . Roberts, S. W. (2007). Children With Autism: Effect of Iron Supplementation on Sleep and Ferritin. *Pediatr Neurol, 36*(3), 152-158. doi:https://doi.org/10.1016/j.pediatrneurol.2006.11.004

Dowd, K. P., Szeklicki, R., Minetto, M. A., Murphy, M. H., Polito, A., Ghigo, E., . . . Donnelly, A. E. (2018). A systematic literature review of reviews on techniques for physical activity measurement in adults: a DEDIPAC study. *International Journal of Behavioral Nutrition and Physical Activity, 15*(1), 15. doi:10.1186/s12966-017-0636-2

Du, G., Liu, T., Lewis, M. M., Kong, L., Wang, Y., Connor, J., . . . Huang, X. (2016). Quantitative susceptibility mapping of the midbrain in Parkinson's disease. *Movement Disorders, 31*(3), 317-324.

Duncan, A., Meek, J. H., Clemence, M., Elwell, C. E., Fallon, P., Tyszczuk, L., . . . Delpy, D. T. (1996). Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatr Res, 39*(5), 889-894. doi:10.1203/00006450-199605000-00025

Duncan, A., Meek, J. H., Clemence, M., Elwell, C. E., Tyszczuk, L., Cope, M., & Delpy, D. T. (1995). Optical pathlength measurements on adult head, calf and forearm and the

head of the newborn infant using phase resolved optical spectroscopy. *Phys Med Biol, 40*(2), 295-304. doi:10.1088/0031-9155/40/2/007

Duque, X., Martinez, H., Vilchis-Gil, J., Mendoza, E., Flores-Hernández, S., Morán, S., . . . Mera, R. M. (2014). Effect of supplementation with ferrous sulfate or iron bis-glycinate chelate on ferritin concentration in Mexican schoolchildren: a randomized controlled trial. *Nutrition journal, 13*, 71-71. doi:10.1186/1475-2891-13-71

Dziembowska, I., Kwapisz, J., Izdebski, P., & Żekanowska, E. (2019). Mild iron deficiency may affect female endurance and behavior. *Physiology & Behavior, 205*, 44-50. doi:https://doi.org/10.1016/j.physbeh.2018.09.012

Dzirasa, K., Ribeiro, S., Costa, R., Santos, L. M., Lin, S.-C., Grosmark, A., . . . Nicolelis, M. A. L. (2006). Dopaminergic control of sleep-wake states. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 26*(41), 10577-10589. doi:10.1523/JNEUROSCI.1767-06.2006

Ehn, L., Carlmark, B., & Hoglund, S. (1980). Iron status in athletes involved in intense physical activity. *Med Sci Sports Exerc, 12*(1), 61-64.

Ekiz, C., Agaoglu, L., Karakas, Z., Gurel, N., & Yalcin, I. (2005). The effect of iron deficiency anemia on the function of the immune system. *Hematol J, 5*(7), 579-583. doi:10.1038/sj.thj.6200574

El Hangouche, A. J., Jniene, A., Aboudrar, S., Errguig, L., Rkain, H., Cherti, M., & Dakka, T. (2018). Relationship between poor quality sleep, excessive daytime sleepiness and low academic performance in medical students. *Adv Med Educ Pract, 9*, 631-638. doi:10.2147/amep.S162350

Elema, T., Yimam, K., Waka, F., & Olana, B. (2018). Folate and Vitamin B-12 status of anemic pregnant women and association to hemoglobin during antenatal care, 17-37 weeks in Ambo Hospital, Oromia, Ethiopia, a multi regression analysis of socio-economic and serum folate and Vitamin B-12. *Journal of Nutrition and Human Health, 02*. doi:10.35841/nutrition-human-health.2.1.28-34

Ellingson, L. D., Kuffel, A. E., Vack, N. J., & Cook, D. B. (2014). Active and sedentary behaviors influence feelings of energy and fatigue in women. *Med Sci Sports Exerc, 46*(1), 192-200. doi:10.1249/MSS.0b013e3182a036ab

Elwood, P. C., & Hughes, D. (1970). Clinical trial of iron therapy of psychomotor function in anaemic women. *Br Med J, 3*(5717), 254-255. doi:10.1136/bmj.3.5717.254

Ems, T., & Huecker, M. R. (2020). Biochemistry, Iron Absorption. In *StatPearls*. Treasure Island (FL): StatPearls Publishing

StatPearls Publishing LLC.

Engberg, I., Segerstedt, J., Waller, G., Wennberg, P., & Eliasson, M. (2017). Fatigue in the general population- associations to age, sex, socioeconomic status, physical activity,

sitting time and self-rated health: the northern Sweden MONICA study 2014. *BMC Public Health, 17*(1), 654. doi:10.1186/s12889-017-4623-y

Erikson, K. M., Jones, B. C., & Beard, J. L. (2000). Iron Deficiency Alters Dopamine Transporter Functioning in Rat Striatum. *The Journal of nutrition, 130*(11), 2831-2837. doi:10.1093/jn/130.11.2831

Erikson, K. M., Jones, B. C., Hess, E. J., Zhang, Q., & Beard, J. L. (2001). Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. *Pharmacol Biochem Behav, 69*(3-4), 409-418. doi:10.1016/s0091-3057(01)00563-9

Eschle, T. M., Goodall, S., Kennedy, D. O., & Wightman, E. L. (2019). Acute Resveratrol Administration Increases Neural Effort but Not Whole Body Metabolism or Cognitive Performance in Healthy, Young Participants. *Journal of Cognitive Enhancement*. doi:10.1007/s41465-019-00139-2

Espie, C. A., Kyle, S. D., Hames, P., Gardani, M., Fleming, L., & Cape, J. (2014). The Sleep Condition Indicator: a clinical screening tool to evaluate insomnia disorder. *BMJ Open, 4*(3), e004183. doi:10.1136/bmjopen-2013-004183

Etebary, S., Nikseresht, S., Sadeghipour, H. R., & Zarrindast, M. R. (2010). Postpartum depression and role of serum trace elements. *Iran J Psychiatry, 5*(2), 40-46.

Evangelou, C., Kartakoullis, N., Hadjicharalambous, M., Aphamis, G., Hadjimarkou, M., Sakkas, G. K., & Giannaki, C. D. (2019). Depressive symptoms, sleep quality, physical fitness, and fatigue among adult women with different obesity status. *Sport Sciences for Health*, 1-10.

Evstatiev, R., Bukaty, A., Jimenez, K., Kulnigg-Dabsch, S., Surman, L., Schmid, W., . . . Gasche, C. (2014). Iron deficiency alters megakaryopoiesis and platelet phenotype independent of thrombopoietin. *Am J Hematol, 89*(5), 524-529. doi:10.1002/ajh.23682

Fairbank, V. (2001). Iron deficiency. *Williams hematology*, 447-470.

Fairclough, S. H., Venables, L., & Tattersall, A. (2005). The influence of task demand and learning on the psychophysiological response. *Int J Psychophysiol, 56*(2), 171-184. doi:10.1016/j.ijpsycho.2004.11.003

Fairweather-Tait, S. J., Wawer, A. A., Gillings, R., Jennings, A., & Myint, P. K. (2014). Iron status in the elderly. *Mechanisms of ageing and development, 136-137*(100), 22-28. doi:10.1016/j.mad.2013.11.005

Falkingham, M., Abdelhamid, A., Curtis, P., Fairweather-Tait, S., Dye, L., & Hooper, L. (2010). The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta-analysis. *Nutrition journal, 9*, 4-4. doi:10.1186/1475-2891-9-4

Fantini, S., & Sassaroli, A. (2020). Frequency-Domain Techniques for Cerebral and Functional Near-Infrared Spectroscopy. *Frontiers in neuroscience, 14*, 300-300. doi:10.3389/fnins.2020.00300

Fatima, Y., Doi, S. A. R., Najman, J. M., & Mamun, A. A. (2016). Exploring Gender Difference in Sleep Quality of Young Adults: Findings from a Large Population Study. *Clinical medicine & research, 14*(3-4), 138-144. doi:10.3121/cmr.2016.1338

Favrat, B., Balck, K., Breymann, C., Hedenus, M., Keller, T., Mezzacasa, A., & Gasche, C. (2014). Evaluation of a single dose of ferric carboxymaltose in fatigued, iron-deficient women–PREFER a randomized, placebo-controlled study. *PLoS One, 9*(4), e94217.

Fayet, F., Flood, V., Petocz, P., & Samman, S. (2014). Avoidance of meat and poultry decreases intakes of omega-3 fatty acids, vitamin B12 , selenium and zinc in young women. *J Hum Nutr Diet, 27 Suppl 2*, 135-142. doi:10.1111/jhn.12092

Fearnley, S. (1997). MRC Psycholinguistic Database search program. *Behavior Research Methods, Instruments, & Computers, 29*(2), 291-295. doi:10.3758/BF03204829

Feng, J., Pratt, J., & Spence, I. (2012). Attention and visuospatial working memory share the same processing resources. *Frontiers in Psychology, 3*. doi:10.3389/fpsyg.2012.00103

Feng, X. B., Yang, X. Q., & Shen, J. (1994). Influence of iron deficiency on serum IgG subclass and pneumococcal polysaccharides specific IgG subclass antibodies. *Chin Med J (Engl), 107*(11), 813-816.

Ferrara, G., Kim, J., Lin, S., Hua, J., & Seto, E. (2019). A Focused Review of Smartphone Diet-Tracking Apps: Usability, Functionality, Coherence With Behavior Change Theory, and Comparative Validity of Nutrient Intake and Energy Estimates. *JMIR Mhealth Uhealth, 7*(5), e9232. doi:10.2196/mhealth.9232

Ferreira, A., Neves, P., & Gozzelino, R. (2019). Multilevel Impacts of Iron in the Brain: The Cross Talk between Neurophysiological Mechanisms, Cognition, and Social Behavior. *Pharmaceuticals (Basel, Switzerland), 12*(3), 126. doi:10.3390/ph12030126

Fiddler, J., Seymour, J., Hernandez-Cordero, S., Campos, I., & Haas, J. (2019). Iron Supplementation Improves Energetic Efficiency During Submaximal Exercise in Iron Deficient Non-anemic Women (P24-042-19). *Current Developments in Nutrition, 3*(Supplement_1). doi:10.1093/cdn/nzz044.P24-042-19

Figueroa-Méndez, R., & Rivas-Arancibia, S. (2015). Vitamin C in Health and Disease: Its Role in the Metabolism of Cells and Redox State in the Brain. *Front Physiol, 6*, 397-397. doi:10.3389/fphys.2015.00397

Finch, C., Miller, L., Inamdar, A., Person, R., Seiler, K., & Mackler, B. (1976). Iron deficiency in the rat. Physiological and biochemical studies of muscle dysfunction. *J Clin Invest, 58*(2), 447-453.

Fisher, A. E. O., & Naughton, D. P. (2004). Iron supplements: the quick fix with long-term consequences. *Nutrition journal, 3*, 2-2. doi:10.1186/1475-2891-3-2

Fisher, A. L., & Nemeth, E. (2017). Iron homeostasis during pregnancy. *The American journal of clinical nutrition, 106*(Suppl 6), 1567S-1574S. doi:10.3945/ajcn.117.155812

Foley, D., Hay, D. A., & Mitchell, R. J. (1986). Specific cognitive effects of mild iron deficiency and associations with blood polymorphisms in young adults. *Annals of Human Biology, 13*(5), 417-425. doi:10.1080/03014468600008601

Fordy, J., & Benton, D. (1994). Does low iron status influence psychological functioning? *Journal of Human Nutrition and Dietetics, 7*(2), 127-133. doi:10.1111/j.1365-277X.1994.tb00420.x

Forestell, C. A., & Nezlek, J. B. (2018). Vegetarianism, depression, and the five factor model of personality. *Ecol Food Nutr, 57*(3), 246-259. doi:10.1080/03670244.2018.1455675

Fouad, G. T., Evans, M., Sharma, P., Baisley, J., Crowley, D., & Guthrie, N. (2013). A randomized, double-blind clinical study on the safety and tolerability of an iron multi-amino acid chelate preparation in premenopausal women. *J Diet Suppl, 10*(1), 17-28. doi:10.3109/19390211.2012.758217

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol, 55*(2), 628-634. doi:10.1152/jappl.1983.55.2.628

Fredriksson, A., Schröder, N., Eriksson, P., Izquierdo, I., & Archer, T. (1999). Neonatal Iron Exposure Induces Neurobehavioural Dysfunctions in Adult Mice. *Toxicology and Applied Pharmacology, 159*(1), 25-30. doi:https://doi.org/10.1006/taap.1999.8711

Fretham, S. J., Carlson, E. S., & Georgieff, M. K. (2011). The role of iron in learning and memory. *Adv Nutr, 2*(2), 112-121. doi:10.3945/an.110.000190

Friedman, A., Chen, Z., Ford, P., Johnson, C., López, A. M., Shander, A., . . . Van Wyck, D. (2012). Iron Deficiency Anemia in Women Across the Life Span. *Journal of women's health (2002), 21*, 1282-1289. doi:10.1089/jwh.2012.3713

Friel, J. K., Aziz, K., Andrews, W. L., Harding, S. V., Courage, M. L., & Adams, R. J. (2003). A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J Pediatr, 143*(5), 582-586. doi:10.1067/s0022-3476(03)00301-9

Fukushima, T., Nakano, J., Ishii, S., Natsuzako, A., Kawachi, H., Sakamoto, J., . . . Okita, M. (2019). Influence of Hemoglobin Level on Muscle and Physical Functions, Activities of Daily Living, and Quality of Life in Patients With Hematological Malignancies.

Integrative cancer therapies, 18, 1534735419842196-1534735419842196. doi:10.1177/1534735419842196

Furukawa, T., Naitoh, Y., Kohno, H., Tokunaga, R., & Taketani, S. (1992). Iron deprivation decreases ribonucleotide reductase activity and DNA synthesis. *Life Sci, 50*(26), 2059-2065. doi:10.1016/0024-3205(92)90572-7

Gaffney-Stomberg, E., & McClung, J. P. (2012). Inflammation and diminished iron status: mechanisms and functional outcomes. *Curr Opin Clin Nutr Metab Care, 15*(6), 605-613. doi:10.1097/MCO.0b013e328357f63b

Gahagan, S., Delker, E., Blanco, E., Burrows, R., & Lozoff, B. (2019). Randomized Controlled Trial of Iron-Fortified versus Low-Iron Infant Formula: Developmental Outcomes at 16 Years. *The Journal of Pediatrics, 212*, 124-130.e121. doi:10.1016/j.jpeds.2019.05.030

Galioto, R., & Spitznagel, M. B. (2016). The Effects of Breakfast and Breakfast Composition on Cognition in Adults. *Adv Nutr, 7*(3), 576s-589s. doi:10.3945/an.115.010231

Gallego, E., Barcos, V., Correa, E., Sánchez Espinosa, E., & Callejo, A. (2016). Influence of the Perceived Workload of Students on the Academic Performance Rates. *International Journal of Engineering Education, 32*, 670-681.

Gandek, B., Ware, J. E., Aaronson, N. K., Apolone, G., Bjorner, J. B., Brazier, J. E., . . . Sullivan, M. (1998). Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment. *J Clin Epidemiol, 51*(11), 1171-1178. doi:10.1016/s0895-4356(98)00109-7

Garcia-Casal, M. N., Pasricha, S. R., Martinez, R. X., Lopez-Perez, L., & Peña-Rosas, J. P. (2018). Are Current Serum and Plasma Ferritin Cut-offs for Iron Deficiency and Overload Accurate and Reflecting Iron Status? A Systematic Review. *Arch Med Res, 49*(6), 405-417. doi:10.1016/j.arcmed.2018.12.005

Garcia-Casal, M.N., Peña-Rosas, J. P., Urrechaga, E., Escanero, J. F., Huo, J., Martinez, R. X., & Lopez-Perez, L. (2018). Performance and comparability of laboratory methods for measuring ferritin concentrations in human serum or plasma: A systematic review and meta-analysis. PloS one, 13(5), e0196576.Gariballa, S. (2014). Poor vitamin C status is associated with increased depression symptoms following acute illness in older people. *Int J Vitam Nutr Res, 84*(1-2), 12-17. doi:10.1024/0300-9831/a000188

Gemming, L., Utter, J., & Ni Mhurchu, C. (2015). Image-assisted dietary assessment: a systematic review of the evidence. *J Acad Nutr Diet, 115*(1), 64-77. doi:10.1016/j.jand.2014.09.015

Geng, F., Mai, X., Zhan, J., Xu, L., Zhao, Z., Georgieff, M., . . . Lozoff, B. (2015). Impact of Fetal-Neonatal Iron Deficiency on Recognition Memory at 2 Months of Age. *J Pediatr, 167*(6), 1226-1232. doi:10.1016/j.jpeds.2015.08.035

Georgieff, M. K. (2011). Long-term brain and behavioral consequences of early iron deficiency. *Nutrition reviews, 69 Suppl 1*(Suppl 1), S43-S48. doi:10.1111/j.1753-4887.2011.00432.x

Georgieff, M. K., Brunette, K. E., & Tran, P. V. (2015). Early life nutrition and neural plasticity. *Development and psychopathology, 27*(2), 411-423. doi:10.1017/S0954579415000061

Georgieff, M. K., Krebs, N. F., & Cusick, S. E. (2019). The Benefits and Risks of Iron Supplementation in Pregnancy and Childhood. *Annu Rev Nutr, 39*, 121-146. doi:10.1146/annurev-nutr-082018-124213

Gibney, S. M., & Drexhage, H. A. (2013). Evidence for a dysregulated immune system in the etiology of psychiatric disorders. *J Neuroimmune Pharmacol, 8*(4), 900-920. doi:10.1007/s11481-013-9462-8

Gibson-Smith, D., Bot, M., Brouwer, I. A., Visser, M., & Penninx, B. W. J. H. (2018). Diet quality in persons with and without depressive and anxiety disorders. *Journal of Psychiatric Research, 106*, 1-7. doi:https://doi.org/10.1016/j.jpsychires.2018.09.006

Gimeno, D., Marmot, M. G., & Singh-Manoux, A. (2008). Inflammatory markers and cognitive function in middle-aged adults: the Whitehall II study. *Psychoneuroendocrinology, 33*(10), 1322-1334.

Giorgini, E., Fisberg, M., De Paula, R. A., Ferreira, A. M., Valle, J., & Braga, J. A. (2001). The use of sweet rolls fortified with iron bis-glycinate chelate in the prevention of iron deficiency anemia in preschool children. *Arch Latinoam Nutr, 51*(1 Suppl 1), 48-53.

Glahn, D. C., Robinson, J. L., Tordesillas-Gutierrez, D., Monkul, E. S., Holmes, M. K., Green, M. J., & Bearden, C. E. (2010). Fronto-temporal dysregulation in asymptomatic bipolar I patients: A paired associate functional MRI study. *Human brain mapping, 31*(7), 1041-1051.

Global Burden of Disease Pediatrics, C., Kyu, H. H., Pinho, C., Wagner, J. A., Brown, J. C., Bertozzi-Villa, A., . . . Vos, T. (2016). Global and National Burden of Diseases and Injuries Among Children and Adolescents Between 1990 and 2013: Findings From the Global Burden of Disease 2013 Study. *JAMA Pediatr, 170*(3), 267-287. doi:10.1001/jamapediatrics.2015.4276

Goddard, A. F., James, M. W., McIntyre, A. S., & Scott, B. B. (2011). Guidelines for the management of iron deficiency anaemia. *Gut, 60*(10), 1309-1316. doi:10.1136/gut.2010.228874

Golfeyz, S., Lewis, S., & Weisberg, I. S. (2018). Hemochromatosis: pathophysiology, evaluation, and management of hepatic iron overload with a focus on MRI. *Expert Rev Gastroenterol Hepatol, 12*(8), 767-778. doi:10.1080/17474124.2018.1496016

Goodnough, L. T., Nemeth, E., & Ganz, T. (2010). Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood, 116*(23), 4754-4761. doi:10.1182/blood-2010-05-286260

Gottlieb, Y., Truman, M., Cohen, L. A., Leichtmann-Bardoogo, Y., & Meyron-Holtz, E. G. (2012). Endoplasmic reticulum anchored heme-oxygenase 1 faces the cytosol. *Haematologica, 97*(10), 1489-1493. doi:10.3324/haematol.2012.063651

Gozzard, D. (2011). When is high-dose intravenous iron repletion needed? Assessing new treatment options. *Drug Des Devel Ther, 5*, 51-60. doi:10.2147/dddt.S15817

Grantham-McGregor, S., & Ani, C. (2001). A Review of Studies on the Effect of Iron Deficiency on Cognitive Development in Children. *The Journal of nutrition, 131*(2), 649S-668S. doi:10.1093/jn/131.2.649S

Grantham-McGregor, S., & Baker-Henningham, H. (2010). Iron Deficiency in Childhood: Causes and Consequences for Child Development. *Annales Nestlé (English ed.), 68*(3), 105-119. doi:10.1159/000319670

Greig, A. J., Patterson, A. J., Collins, C. E., & Chalmers, K. A. (2013). Iron deficiency, cognition, mental health and fatigue in women of childbearing age: a systematic review. *J Nutr Sci, 2*, e14. doi:10.1017/jns.2013.7

Greminger, A. R., Lee, D. L., Shrager, P., & Mayer-Proschel, M. (2014). Gestational iron deficiency differentially alters the structure and function of white and gray matter brain regions of developing rats. *J Nutr, 144*(7), 1058-1066. doi:10.3945/jn.113.187732

Groner, J. A., Holtzman, N. A., Charney, E., & Mellits, E. D. (1986). A randomized trial of oral iron on tests of short-term memory and attention span in young pregnant women. *J Adolesc Health Care, 7*(1), 44-48. doi:10.1016/s0197-0070(86)80094-8

Grønli, O., Kvamme, J.-M., Friborg, O., & Wynn, R. (2013). Zinc Deficiency Is Common in Several Psychiatric Disorders. *PloS one, 8*, e82793. doi:10.1371/journal.pone.0082793

Guerin, S. A., Robbins, C. A., Gilmore, A. W., & Schacter, D. L. (2012). Interactions between visual attention and episodic retrieval: dissociable contributions of parietal regions during gist-based false recognition. *Neuron, 75*(6), 1122-1134. doi:10.1016/j.neuron.2012.08.020

Guglani, L., Gopal, R., Rangel-Moreno, J., Junecko, B. F., Lin, Y., Berger, T., . . . Khader, S. A. (2012). Lipocalin 2 regulates inflammation during pulmonary mycobacterial infections. *PLoS One, 7*(11), e50052. doi:10.1371/journal.pone.0050052

Guo, N., Robakis, T., Miller, C., & Butwick, A. (2018). Prevalence of Depression Among Women of Reproductive Age in the United States. *Obstet Gynecol, 131*(4), 671-679. doi:10.1097/aog.0000000000002535

Guo, S., Huang, J., Jiang, H., Han, C., Li, J., Xu, X., . . . Wang, T. (2017). Restless Legs Syndrome: From Pathophysiology to Clinical Diagnosis and Management. *Frontiers in aging neuroscience, 9*, 171-171. doi:10.3389/fnagi.2017.00171

Gupta, P., Tiwari, S., & Haria, J. (2014). Relationship between depression and vitamin C status: a study on rural patients from western uttar pradesh in India. *Int. J. Sci. Study, 1*, 37-39.

Gupta, P. M., Perrine, C. G., Mei, Z., & Scanlon, K. S. (2017). Correction: Gupta, P.M.; et al. Iron, Anemia, and Iron Deficiency Anemia among Young Children in the United States Nutrients 2016, 8, 330. *Nutrients, 9*(8). doi:10.3390/nu9080876

Guthold, R., Stevens, G. A., Riley, L. M., & Bull, F. C. (2018). Worldwide trends in insufficient physical activity from 2001 to 2016: a pooled analysis of 358 population-based surveys with 1·9 million participants. *The Lancet Global Health, 6*(10), e1077-e1086. doi:10.1016/S2214-109X(18)30357-7

Ha, J-H., Doguer, C., Wang, X., Flores, S.R., & Collins, J.F. (2016). High-Iron Consumption Impairs Growth and Causes Copper-Deficiency Anemia in Weanling Sprague-Dawley Rats. *PLoS One, 11*(8), e0161033

Haas, J., Seymour, J., Hernandez, S., Dehaene, J., & Villalpando, S. (2002). *Iron depletion increases the energy cost of work in non-anemic Mexican women.* Paper presented at the AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY.

Haas, J. D., & Brownlie IV, T. (2001). Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *The Journal of nutrition, 131*(2), 676S-690S.

Haider, L. M., Schwingshackl, L., Hoffmann, G., & Ekmekcioglu, C. (2018). The effect of vegetarian diets on iron status in adults: A systematic review and meta-analysis. *Crit Rev Food Sci Nutr, 58*(8), 1359-1374. doi:10.1080/10408398.2016.1259210

Halis, H., Bor-Kucukatay, M., Akin, M., Kucukatay, V., Bozbay, I., & Polat, A. (2009). Hemorheological parameters in children with iron-deficiency anemia and the alterations in these parameters in response to iron replacement. *Pediatr Hematol Oncol, 26*(3), 108-118. doi:10.1080/08880010902754909

Hallberg, L., Bengtsson, C., Lapidus, L., Lindstedt, G., Lundberg, P. A., & Hulten, L. (1993). Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *Br J Haematol, 85*(4), 787-798. doi:10.1111/j.1365-2141.1993.tb03225.x

Hamid Jan, J. M., Jr., Amal, K. M., Rohani, A., & Norimah, A. K. (2010). Association of Iron Deficiency with or without Anaemia and Cognitive Functions among Primary School Children in Malaysia. *Malays J Nutr, 16*(2), 261-270.

Hamilton, J. P., Sacchet, M. D., Hjørnevik, T., Chin, F. T., Shen, B., Kämpe, R., . . . Gotlib, I. H. (2018). Striatal dopamine deficits predict reductions in striatal functional connectivity in major depression: a concurrent 11C-raclopride positron emission tomography and functional magnetic resonance imaging investigation. *Translational Psychiatry, 8*(1), 264. doi:10.1038/s41398-018-0316-2

Hampl, J. S., Taylor, C. A., & Johnston, C. S. (2004). Vitamin C deficiency and depletion in the United States: the Third National Health and Nutrition Examination Survey, 1988 to 1994. *American journal of public health, 94*(5), 870-875. doi:10.2105/ajph.94.5.870

Hansen, S. N., Tveden-Nyborg, P., & Lykkesfeldt, J. (2014). Does vitamin C deficiency affect cognitive development and function? *Nutrients, 6*(9), 3818-3846.

Hare, D. J., Arora, M., Jenkins, N. L., Finkelstein, D. I., Doble, P. A., & Bush, A. I. (2015). Is early-life iron exposure critical in neurodegeneration? *Nature Reviews Neurology, 11*(9), 536.

Hare, G. M. (2004). Anaemia and the brain. *Curr Opin Anaesthesiol, 17*(5), 363-369. doi:10.1097/00001503-200410000-00003

Hart, S. G. (2006). Nasa-Task Load Index (NASA-TLX); 20 Years Later. *Proceedings of the Human Factors and Ergonomics Society Annual Meeting, 50*(9), 904-908. doi:10.1177/154193120605000909

Hart, S. G., & Staveland, L. E. (1988) Development of NASA-TLX (Task Load Index): Results of Empirical and Theoretical Research. In*: Vol. 52. Advances in Psychology* (pp. 139-183).

Hartkopf, J., Schleger, F., Keune, J., Wiechers, C., Pauluschke-Froehlich, J., Weiss, M., . . . Kiefer-Schmidt, I. (2018). Impact of Intrauterine Growth Restriction on Cognitive and Motor Development at 2 Years of Age. *Front Physiol, 9*, 1278-1278. doi:10.3389/fphys.2018.01278

Harvey, L. J., Armah, C. N., Dainty, J. R., Foxall, R. J., John Lewis, D., Langford, N. J., & Fairweather-Tait, S. J. (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *Br J Nutr, 94*(4), 557-564. doi:10.1079/bjn20051493

Harvey, L. J., Berti, C., Casgrain, A., Cetin, I., Collings, R., Gurinovic, M., . . . Fairweather-Tait, S. J. (2013). EURRECA-Estimating iron requirements for deriving dietary reference values. *Crit Rev Food Sci Nutr, 53*(10), 1064-1076. doi:10.1080/10408398.2012.742860

Haskell, C. F., Robertson, B., Jones, E., Forster, J., Jones, R., Wilde, A., . . . Kennedy, D. O. (2010). Effects of a multi-vitamin/mineral supplement on cognitive function and fatigue during extended multi-tasking. *Hum Psychopharmacol, 25*(6), 448-461. doi:10.1002/hup.1144

Haskell-Ramsay, C. F., Jackson, P. A., Forster, J. S., Dodd, F. L., Bowerbank, S. L., & Kennedy, D. O. (2018). The Acute Effects of Caffeinated Black Coffee on Cognition and Mood in Healthy Young and Older Adults. *Nutrients, 10*(10). doi:10.3390/nu10101386

Haskell-Ramsay, C. F., Stuart, R. C., Okello, E. J., & Watson, A. W. (2017). Cognitive and mood improvements following acute supplementation with purple grape juice in healthy young adults. *European journal of nutrition, 56*(8), 2621-2631. doi:10.1007/s00394-017-1454-7

Hassan, T. H., Badr, M. A., Karam, N. A., Zkaria, M., El Saadany, H. F., Abdel Rahman, D. M., . . . Selim, A. M. (2016). Impact of iron deficiency anemia on the function of the immune system in children. *Medicine, 95*(47), e5395-e5395. doi:10.1097/MD.0000000000005395

Hauer, B. E., Negash, B., Chan, K., Vuong, W., Colbourne, F., Pagliardini, S., & Dickson, C. T. (2018). Hyperoxia enhances slow-wave forebrain states in urethane-anesthetized and naturally sleeping rats. *Journal of Neurophysiology, 120*(4), 1505-1515. doi:10.1152/jn.00373.2018

Hay, G., Refsum, H., Whitelaw, A., Melbye, E. L., Haug, E., & Borch-Iohnsen, B. (2007). Predictors of serum ferritin and serum soluble transferrin receptor in newborns and their associations with iron status during the first 2 y of life. *The American journal of clinical nutrition, 86*(1), 64-73. doi:10.1093/ajcn/86.1.64

Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr, 4*(2), 197-206. doi:10.1079/phn200054

Hegde, N., Rich, M. W., & Gayomali, C. (2006). The cardiomyopathy of iron deficiency. *Texas Heart Institute journal, 33*(3), 340-344. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17041692

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1592266/

Henderson, S. A., Dallman, P. R., & Brooks, G. A. (1986). Glucose turnover and oxidation are increased in the iron-deficient anemic rat. *Am J Physiol, 250*(4 Pt 1), E414-421. doi:10.1152/ajpendo.1986.250.4.E414

Hennigar, S. R. (2019). Ironing out the Relation between Iron Supplementation and Exercise Performance in the Absence of Anemia. *The Journal of nutrition, 149*(2), 177-178. doi:10.1093/jn/nxy288

Hermoso, M., Vucic, V., Vollhardt, C., Arsic, A., Roman-Vinas, B., Iglesia-Altaba, I., . . . Koletzko, B. (2011). The effect of iron on cognitive development and function in infants, children and adolescents: a systematic review. *Ann Nutr Metab, 59*(2-4), 154-165. doi:10.1159/000334490

Hidese, S., Saito, K., Asano, S., & Kunugi, H. (2018). Association between iron-deficiency anemia and depression: A web-based Japanese investigation. *Psychiatry and Clinical Neurosciences, 72*(7), 513-521. doi:10.1111/pcn.12656

Hinton, P. S., Giordano, C., Brownlie, T., & Haas, J. D. (2000). Iron supplementation improves endurance after training in iron-depleted, nonanemic women. *Journal of Applied Physiology, 88*(3), 1103-1111.

Hinton, P. S., & Sinclair, L. M. (2007). Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. *European Journal of Clinical Nutrition, 61*(1), 30-39. doi:10.1038/sj.ejcn.1602479

Hoes, M. F., Grote Beverborg, N., Kijlstra, J. D., Kuipers, J., Swinkels, D. W., Giepmans, B. N. G., . . . van der Meer, P. (2018). Iron deficiency impairs contractility of human cardiomyocytes through decreased mitochondrial function. *European journal of heart failure, 20*(5), 910-919. doi:10.1002/ejhf.1154

Hojman, P., Brolin, C., Nørgaard-Christensen, N., Dethlefsen, C., Lauenborg, B., Olsen, C. K., . . . Pedersen, B. K. (2019). IL-6 release from muscles during exercise is stimulated by lactate-dependent protease activity. *American Journal of Physiology-Endocrinology and Metabolism, 316*(5), E940-E947. doi:10.1152/ajpendo.00414.2018

Hong, C. T., Huang, Y. H., Liu, H. Y., Chiou, H.-Y., Chan, L., & Chien, L.-N. (2016). Newly Diagnosed Anemia Increases Risk of Parkinson's disease: A Population-Based Cohort Study. *Scientific reports, 6*, 29651-29651. doi:10.1038/srep29651

Hoshi, Y. (2007). Functional near-infrared spectroscopy: current status and future prospects. *J Biomed Opt, 12*(6), 062106. doi:10.1117/1.2804911

Houston, B. L., Hurrie, D., Graham, J., Perija, B., Rimmer, E., Rabbani, R., . . . Zarychanski, R. (2018). Efficacy of iron supplementation on fatigue and physical capacity in non-anaemic iron-deficient adults: a systematic review of randomised controlled trials. *BMJ Open, 8*(4), e019240. doi:10.1136/bmjopen-2017-019240

Huang, J. (2019). Greater brain activity during the resting state and the control of activation during the performance of tasks. *Scientific reports, 9*(1), 5027. doi:10.1038/s41598-019-41606-2

Hyder, F., Rothman, D. L., & Bennett, M. R. (2013). Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species

and activity levels. *Proc Natl Acad Sci U S A, 110*(9), 3549-3554. doi:10.1073/pnas.1214912110

Iglesias, L., Canals, J., & Arija, V. (2018). Effects of prenatal iron status on child neurodevelopment and behavior: A systematic review. *Crit Rev Food Sci Nutr, 58*(10), 1604-1614. doi:10.1080/10408398.2016.1274285

Iglesias Vázquez, L., Canals, J., Voltas, N., Jardí, C., Hernández, C., Bedmar, C., . . . Arija, V. (2019). Does the fortified milk with high iron dose improve the neurodevelopment of healthy infants? Randomized controlled trial. *BMC Pediatrics, 19*(1), 315. doi:10.1186/s12887-019-1679-0

Ilyas, S., & Moncrieff, J. (2012). Trends in prescriptions and costs of drugs for mental disorders in England, 1998-2010. *Br J Psychiatry, 200*(5), 393-398. doi:10.1192/bjp.bp.111.104257

Insel, B. J., Schaefer, C. A., McKeague, I. W., Susser, E. S., & Brown, A. S. (2008). Maternal iron deficiency and the risk of schizophrenia in offspring. *Arch Gen Psychiatry, 65*(10), 1136-1144. doi:10.1001/archpsyc.65.10.1136

Ishibashi, A., Maeda, N., Sumi, D., & Goto, K. (2017). Elevated Serum Hepcidin Levels during an Intensified Training Period in Well-Trained Female Long-Distance Runners. *Nutrients, 9*(3). doi:10.3390/nu9030277

Iyoke, C. A., Emegoakor, F. C., Ezugwu, E. C., Lawani, L. O., Ajah, L. O., Madu, J. A., . . . Ezugwu, F. O. (2017). Effect of treatment with single total-dose intravenous iron versus daily oral iron(III)-hydroxide polymaltose on moderate puerperal iron-deficiency anemia. *Ther Clin Risk Manag, 13*, 647-653. doi:10.2147/tcrm.S112227

Jacka, F. N., Pasco, J. A., Williams, L. J., Mann, N., Hodge, A., Brazionis, L., & Berk, M. (2012). Red Meat Consumption and Mood and Anxiety Disorders. *Psychotherapy and Psychosomatics, 81*(3), 196-198. doi:10.1159/000334910

Jackson, J., Williams, R., McEvoy, M., MacDonald-Wicks, L., & Patterson, A. (2016). Is Higher Consumption of Animal Flesh Foods Associated with Better Iron Status among Adults in Developed Countries? A Systematic Review. *Nutrients, 8*(2), 89-89. doi:10.3390/nu8020089

Jacobs, P., Wood, L., & Bird, A. R. (2000). Erythrocytes: Better Tolerance of Iron Polymaltose Complex Compared with Ferrous Sulphate in the Treatment of Anaemia. *Hematology, 5*(1), 77-83. doi:10.1080/10245332.2000.11746490

Jacobs, P., Wormald, L. A., & Gregory, M. C. (1979). Absorption of iron polymaltose and ferrous sulphate in rats and humans. A comparative study. *S Afr Med J, 55*(26), 1065-1072.

Jaeggi, T., Kortman, G. A., Moretti, D., Chassard, C., Holding, P., Dostal, A., . . . Zimmermann, M. B. (2015). Iron fortification adversely affects the gut microbiome,

increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut, 64*(5), 731-742. doi:10.1136/gutjnl-2014-307720

Jahani, S., Fantana, A. L., Harper, D., Ellison, J. M., Boas, D. A., Forester, B. P., & Yücel, M. A. (2017). fNIRS can robustly measure brain activity during memory encoding and retrieval in healthy subjects. *Scientific Reports, 7*(1), 9533. doi:10.1038/s41598-017-09868-w

Jaime-Perez, J. C., Herrera-Garza, J. L., & Gomez-Almaguer, D. (2005). Sub-optimal fetal iron acquisition under a maternal environment. *Arch Med Res, 36*(5), 598-602. doi:10.1016/j.arcmed.2005.03.023

Jain, A., Chowdhury, N., & Jain, S. (2018). Intra- and inter-model reliability of Hemocue Hb 201+ and Hemocue Hb 301 devices. *Asian J Transfus Sci, 12*(2), 123–126.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., . . . Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet, 392*(10159), 1789-1858. doi:https://doi.org/10.1016/S0140-6736(18)32279-7

Jang, Y., Kim, J. H., & Lee, K. (2017). Validation of the revised piper fatigue scale in Koreans with chronic hepatitis B. *PLoS One, 12*(5), e0177690. doi:10.1371/journal.pone.0177690

Jarosz, P. A., Davis, J. E., Yarandi, H. N., Farkas, R., Feingold, E., Shippings, S. H., . . . Williams, D. (2014). Obesity in urban women: associations with sleep and sleepiness, fatigue and activity. *Womens Health Issues, 24*(4), e447-454. doi:10.1016/j.whi.2014.04.005

Jáuregui-Lobera, I. (2014). Iron deficiency and cognitive functions. *Neuropsychiatric disease and treatment, 10*, 2087-2095. doi:10.2147/NDT.S72491

Jenkins, D. J. A., Spence, J. D., Giovannucci, E. L., Kim, Y.-i., Josse, R., Vieth, R., . . . Sievenpiper, J. L. (2018). Supplemental Vitamins and Minerals for CVD Prevention and Treatment. *Journal of the American College of Cardiology, 71*(22), 2570-2584. doi:https://doi.org/10.1016/j.jacc.2018.04.020

Ji, Y., Flower, R., Hyland, C., Saiepour, N., & Faddy, H. (2018). Genetic factors associated with iron storage in Australian blood donors. *Blood transfusion = Trasfusione del sangue, 16*(2), 123-129. doi:10.2450/2016.0138-16

Jimmy, B., & Jose, J. (2011). Patient medication adherence: measures in daily practice. *Oman medical journal, 26*(3), 155-159. doi:10.5001/omj.2011.38

Jirout, J., LoCasale-Crouch, J., Turnbull, K., Gu, Y., Cubides, M., Garzione, S., . . . Kranz, S. (2019). How Lifestyle Factors Affect Cognitive and Executive Function and the Ability to Learn in Children. *Nutrients, 11*(8), 1953. doi:10.3390/nu11081953

Jobarteh, M. L., McCrory, M. A., Lo, B., Sun, M., Sazonov, E., Anderson, A. K., . . . Frost, G. (2020). Development and Validation of an Objective, Passive Dietary Assessment Method for Estimating Food and Nutrient Intake in Households in Low- and Middle-Income Countries: A Study Protocol. *Current Developments in Nutrition, 4*(2). doi:10.1093/cdn/nzaa020

Johnson, E. E., Srikanth, C. V., Sandgren, A., Harrington, L., Trebicka, E., Wang, L., . . . Cherayil, B. J. (2010). Siderocalin inhibits the intracellular replication of Mycobacterium tuberculosis in macrophages. *FEMS immunology and medical microbiology, 58*(1), 138-145. doi:10.1111/j.1574-695X.2009.00622.x

Johnson, E. E., & Wessling-Resnick, M. (2012). Iron metabolism and the innate immune response to infection. *Microbes and infection, 14*(3), 207-216. doi:10.1016/j.micinf.2011.10.001

Johnson, S., Lang, A., Sturm, M., & O'Brien, S. H. (2016). Iron Deficiency without Anemia: A Common Yet Under-Recognized Diagnosis in Young Women with Heavy Menstrual Bleeding. *J Pediatr Adolesc Gynecol, 29*(6), 628-631. doi:10.1016/j.jpag.2016.05.009

Johnson-Wimbley, T. D., & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Therapeutic advances in gastroenterology, 4*(3), 177-184. doi:10.1177/1756283X11398736

Joint World Health Organization/Centers for Disease, C., & Prevention Technical Consultation on the Assessment of Iron Status at the Population Level. (2007). *Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6-8 April 2004* (2nd ed ed.). Geneva: World Health Organization.

Jones, S., Chiesa, S. T., Chaturvedi, N., & Hughes, A. D. (2016). Recent developments in near-infrared spectroscopy (NIRS) for the assessment of local skeletal muscle microvascular function and capacity to utilise oxygen. *Artery research, 16*, 25-33. doi:10.1016/j.artres.2016.09.001

Jorgenson, L. A., Wobken, J. D., & Georgieff, M. K. (2003). Perinatal iron deficiency alters apical dendritic growth in hippocampal CA1 pyramidal neurons. *Dev Neurosci, 25*(6), 412-420. doi:10.1159/000075667

Jovanovic, H., Lundberg, J., Karlsson, P., Cerin, Å., Saijo, T., Varrone, A., . . . Nordström, A.-L. (2008). Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *NeuroImage, 39*(3), 1408-1419. doi:https://doi.org/10.1016/j.neuroimage.2007.10.016

Kaladhar, M., & Narasinga Rao, B. S. (1982). Effects of iron deficiency on serotonin uptake in vitro by rat brain synaptic vesicles. *J Neurochem, 38*(6), 1576-1581. doi:10.1111/j.1471-4159.1982.tb06635.x

Kamdi, S. P., & Palkar, P. J. (2015). Efficacy and safety of ferrous asparto glycinate in the management of iron deficiency anaemia in pregnant women. *Journal of Obstetrics and Gynaecology, 35*(1), 4-8. doi:10.3109/01443615.2014.930098

Karlsson, T. S., Marions, L. B., & Edlund, M. G. (2014). Heavy menstrual bleeding significantly affects quality of life. *Acta Obstet Gynecol Scand, 93*(1), 52-57. doi:10.1111/aogs.12292

Kassab, A., Le Lan, J., Tremblay, J., Vannasing, P., Dehbozorgi, M., Pouliot, P., . . . Nguyen, D. K. (2018). Multichannel wearable f NIRS-EEG system for long-term clinical monitoring. *Human brain mapping, 39*(1), 7-23.

Kennedy, B. C., Wallin, D. J., Tran, P. V., & Georgieff, M. K. (2016). Long-Term Brain and Behavioral Consequences of Early-Life Iron Deficiency. In N. Reissland & B. S. Kisilevsky (Eds.), *Fetal Development: Research on Brain and Behavior, Environmental Influences, and Emerging Technologies* (pp. 295-316). Cham: Springer International Publishing.

Kennedy, D., Okello, E., Chazot, P., Howes, M. J., Ohiomokhare, S., Jackson, P., . . . Wightman, E. (2018). Volatile Terpenes and Brain Function: Investigation of the Cognitive and Mood Effects of Mentha × Piperita L. Essential Oil with In Vitro Properties Relevant to Central Nervous System Function. *Nutrients, 10*(8). doi:10.3390/nu10081029

Kennedy, D., Wightman, E., Khan, J., Grothe, T., & Jackson, P. (2019). The Acute and Chronic Cognitive and Cerebral Blood-Flow Effects of Nepalese Pepper (Zanthoxylum armatum DC.) Extract-A Randomized, Double-Blind, Placebo-Controlled Study in Healthy Humans. *Nutrients, 11*(12). doi:10.3390/nu11123022

Kennedy, D. O. (2016). B Vitamins and the Brain: Mechanisms, Dose and Efficacy--A Review. *Nutrients, 8*(2), 68. doi:10.3390/nu8020068

Kennedy, D. O., & Haskell, C. F. (2011). Vitamins and cognition: what is the evidence? *Drugs, 71*(15), 1957-1971. doi:10.2165/11594130-000000000-00000

Kennedy, D. O., Haskell, C. F., Robertson, B., Reay, J., Brewster-Maund, C., Luedemann, J., . . . Scholey, A. B. (2008). Improved cognitive performance and mental fatigue following a multi-vitamin and mineral supplement with added guaraná (Paullinia cupana). *Appetite, 50*(2-3), 506-513. doi:10.1016/j.appet.2007.10.007

Kennedy, D. O., & Scholey, A. B. (2004). A glucose-caffeine 'energy drink' ameliorates subjective and performance deficits during prolonged cognitive demand. *Appetite, 42*(3), 331-333. doi:https://doi.org/10.1016/j.appet.2004.03.001

Kennedy, D. O., Stevenson, E. J., Jackson, P. A., Dunn, S., Wishart, K., Bieri, G., . . . Haskell-Ramsay, C. F. (2016). Multivitamins and minerals modulate whole-body energy metabolism and cerebral blood-flow during cognitive task performance: a double-blind, randomised, placebo-controlled trial. *Nutrition & Metabolism, 13*(1), 11. doi:10.1186/s12986-016-0071-4

Kennedy, D. O., Veasey, R., Watson, A., Dodd, F., Jones, E., Maggini, S., & Haskell, C. F. (2010). Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males. *Psychopharmacology (Berl), 211*(1), 55-68. doi:10.1007/s00213-010-1870-3

Kennedy, D. O., Wightman, E. L., Forster, J., Khan, J., Haskell-Ramsay, C. F., & Jackson, P. A. (2017). Cognitive and Mood Effects of a Nutrient Enriched Breakfast Bar in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled, Parallel Groups Study. *Nutrients, 9*(12). doi:10.3390/nu9121332

Kessels, R. P. C., Nys, G. M. S., Brands, A. M. A., van den Berg, E., & Van Zandvoort, M. J. E. (2006). The modified Location Learning Test: Norms for the assessment of spatial memory function in neuropsychological patients. *Archives of Clinical Neuropsychology, 21*(8), 841-846. doi:https://doi.org/10.1016/j.acn.2006.06.015

Khaled, S., Brun, J. F., Wagner, A., Mercier, J., Bringer, J., & Prefaut, C. (1998). Increased blood viscosity in iron-depleted elite athletes. *Clin Hemorheol Microcirc, 18*(4), 309-318.

Khedr, E., Hamed, S. A., Elbeih, E., El-Shereef, H., Ahmad, Y., & Ahmed, S. (2008). Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment. *Eur Arch Psychiatry Clin Neurosci, 258*(8), 489-496. doi:10.1007/s00406-008-0822-y

Khoshfetrat, M. R., Mohammadi, F., Mortazavi, S., Rashidi, A., Neyestani, T., Kalantari, N., & Esmaillzadeh, A. (2013). The effect of iron-vitamin C co-supplementation on biomarkers of oxidative stress in iron-deficient female youth. *Biol Trace Elem Res, 153*(1-3), 171-177. doi:10.1007/s12011-013-9695-7

Kim, C., Nan, B., Kong, S., & Harlow, S. (2012). Changes in iron measures over menopause and associations with insulin resistance. *Journal of women's health (2002), 21*(8), 872-877. doi:10.1089/jwh.2012.3549

Kim, D. R., Epperson, C. N., Weiss, A. R., & Wisner, K. L. (2014). Pharmacotherapy of postpartum depression: an update. *Expert Opin Pharmacother, 15*(9), 1223-1234. doi:10.1517/14656566.2014.911842

Kim, I., Yetley, E. A., & Calvo, M. S. (1993). Variations in iron-status measures during the menstrual cycle. *The American journal of clinical nutrition, 58*(5), 705-709. doi:10.1093/ajcn/58.5.705

Kim, J., & Wessling-Resnick, M. (2014). Iron and mechanisms of emotional behavior. *J Nutr Biochem, 25*(11), 1101-1107. doi:10.1016/j.jnutbio.2014.07.003

Kim, K. M., Hwang, H. R., Kim, Y. J., Lee, J. G., Yi, Y. H., Tak, Y. J., . . . Chung, S. I. (2019). Association between Serum-Ferritin Levels and Sleep Duration, Stress, Depression, and Suicidal Ideation in Older Koreans: Fifth Korea National Health and Nutrition Examination Survey 2010-2012. *Korean journal of family medicine, 40*(6), 380-387. doi:10.4082/kjfm.18.0097

Kim, M., & Park, J.-M. (2017). Factors affecting cognitive function according to gender in community-dwelling elderly individuals. *Epidemiology and health, 39*, e2017054-e2017054. doi:10.4178/epih.e2017054

Kipnis, J., Cohen, H., Cardon, M., Ziv, Y., & Schwartz, M. (2004). T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. *Proc Natl Acad Sci U S A, 101*(21), 8180-8185. doi:10.1073/pnas.0402268101

Klempa, K. L., Willis, W. T., Chengson, R., Dallman, P. R., & Brooks, G. A. (1989). Iron deficiency decreases gluconeogenesis in isolated rat hepatocytes. *J Appl Physiol (1985), 67*(5), 1868-1872. doi:10.1152/jappl.1989.67.5.1868

Klinkenberg, I., Sambeth, A., & Blokland, A. (2011). Acetylcholine and attention. *Behavioural brain research, 221*(2), 430-442.

Knutson, M. D. (2017). Iron transport proteins: Gateways of cellular and systemic iron homeostasis. *Journal of Biological Chemistry, 292*(31), 12735-12743. doi:10.1074/jbc.R117.786632

Kocaoz, S., Cirpan, R., & Degirmencioglu, A. Z. (2019). The prevalence and impacts heavy menstrual bleeding on anemia, fatigue and quality of life in women of reproductive age. *Pakistan journal of medical sciences, 35*(2), 365-370. doi:10.12669/pjms.35.2.644

Kocsis, L., Herman, P., & Eke, A. (2006). The modified Beer-Lambert law revisited. *Phys Med Biol, 51*(5), N91-98. doi:10.1088/0031-9155/51/5/n02

Koehler, K., Braun, H., Achtzehn, S., Hildebrand, U., Predel, H. G., Mester, J., & Schanzer, W. (2012). Iron status in elite young athletes: gender-dependent influences of diet and exercise. *Eur J Appl Physiol, 112*(2), 513-523. doi:10.1007/s00421-011-2002-4

Konofal, E., Lecendreux, M., Deron, J., Marchand, M., Cortese, S., Zaim, M., . . . Arnulf, I. (2008). Effects of iron supplementation on attention deficit hyperactivity disorder in children. *Pediatr Neurol, 38*(1), 20-26. doi:10.1016/j.pediatrneurol.2007.08.014

Kordas, K., & Stoltzfus, R. J. (2004). New evidence of iron and zinc interplay at the enterocyte and neural tissues. *J Nutr, 134*(6), 1295-1298. doi:10.1093/jn/134.6.1295

Korkmaz, S., Yıldız, S., Korucu, T., Gundogan, B., Sunbul, Z. E., Korkmaz, H., & Atmaca, M. (2015). Frequency of anemia in chronic psychiatry patients. *Neuropsychiatric disease and treatment, 11*, 2737-2741. doi:10.2147/NDT.S91581

Korzeniewski, S. J., Allred, E. N., Joseph, R. M., Heeren, T., Kuban, K. C. K., O'Shea, T. M., . . . Investigators, E. S. (2017). Neurodevelopment at Age 10 Years of Children Born <28 Weeks With Fetal Growth Restriction. *Pediatrics, 140*(5), e20170697. doi:10.1542/peds.2017-0697

Kościelniak, B. K., Charchut, A., Wójcik, M., Sztefko, K., & Tomasik, P. J. (2017). Impact of Fasting on Complete Blood Count Assayed in Capillary Blood Samples. *Laboratory Medicine, 48*(4), 357-361. doi:10.1093/labmed/lmx044

Kosinski, M., Ware, J. E., Turner-Bowker, D. M., & Gandek, B. (2007). *User's manual for the SF-12v2 health survey : with a supplement documenting the SF-12Æ health survey*. Lincoln, RI: QualityMetric Incorporated.

Koury, M. J., & Ponka, P. (2004). New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. *Annu Rev Nutr, 24*, 105-131. doi:10.1146/annurev.nutr.24.012003.132306

Kozberg, M., & Hillman, E. (2016). Neurovascular coupling and energy metabolism in the developing brain. *Prog Brain Res, 225*, 213-242. doi:10.1016/bs.pbr.2016.02.002

Kratzer, H. F. (2018). *Chelates in nutrition*: CRC Press.

Krayenbuehl, P.-A., Battegay, E., Breymann, C., Furrer, J., & Schulthess, G. (2011). Intravenous iron for the treatment of fatigue in nonanemic, premenopausal women with low serum ferritin concentration. *Blood, 118*(12), 3222-3227.

Kretsch, M. J., Fong, A. K., Green, M. W., & Johnson, H. L. (1998). Cognitive function, iron status, and hemoglobin concentration in obese dieting women. *Eur J Clin Nutr, 52*(7), 512-518. doi:10.1038/sj.ejcn.1600598

Kuvibidila, S. R., Kitchens, D., & Baliga, B. S. (1999). In vivo and in vitro iron deficiency reduces protein kinase C activity and translocation in murine splenic and purified T cells. *J Cell Biochem, 74*(3), 468-478.

LaChance, L. R., & Ramsey, D. (2018). Antidepressant foods: An evidence-based nutrient profiling system for depression. *World journal of psychiatry, 8*(3), 97-104. doi:10.5498/wjp.v8.i3.97

Laine, F., Angeli, A., Ropert, M., Jezequel, C., Bardou-Jacquet, E., Deugnier, Y., . . . Comets, E. (2016). Variations of hepcidin and iron-status parameters during the menstrual cycle in healthy women. *Br J Haematol, 175*(5), 980-982. doi:10.1111/bjh.13906

LaLumiere, R. T. (2014). 5 - Dopamine and Memory. In A. Meneses (Ed.), *Identification of Neural Markers Accompanying Memory* (pp. 79-94). San Diego: Elsevier.

LaManca, J. J., & Haymes, E. M. (1993). Effects of iron repletion on VO2max, endurance, and blood lactate in women. *Med Sci Sports Exerc, 25*(12), 1386-1392.

Lambert, A., Knaggs, K., Scragg, R., & Schaaf, D. (2002). Effects of iron treatment on cognitive performance and working memory in non-anaemic, iron-deficient girls. *New Zealand Journal of Psychology, 31*(1), 19.

Langkammer, C., Liu, T., Khalil, M., Enzinger, C., Jehna, M., Fuchs, S., . . . Ropele, S. (2013). Quantitative susceptibility mapping in multiple sclerosis. *Radiology, 267*(2), 551-559. doi:10.1148/radiol.12120707

Langkammer, C., Schweser, F., Krebs, N., Deistung, A., Goessler, W., Scheurer, E., . . . Reichenbach, J. R. (2012). Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *NeuroImage, 62*(3), 1593-1599. doi:10.1016/j.neuroimage.2012.05.049

Langley-Evans, S. (2013). *Nutrition: a lifespan approach*: John Wiley & Sons.

Larson, L. M., Phiri, K. S., & Pasricha, S. R. (2017). Iron and Cognitive Development: What Is the Evidence? *Annals of Nutrition and Metabolism, 71(suppl 3)*(Suppl. 3), 25-38. doi:10.1159/000480742

Lavidor, M., Weller, A., & Babkoff, H. (2003). How sleep is related to fatigue. *Br J Health Psychol, 8*(Pt 1), 95-105. doi:10.1348/135910703762879237

Layrisse, M., García-Casal, M. a. N., Solano, L., Barón, M. a. A., Arguello, F., Llovera, D., . . . Tropper, E. (2000). Iron Bioavailability in Humans from Breakfasts Enriched with Iron Bis-Glycine Chelate, Phytates and Polyphenols. *The Journal of nutrition, 130*(9), 2195-2199. doi:10.1093/jn/130.9.2195

Lee, D. L., Strathmann, F. G., Gelein, R., Walton, J., & Mayer-Pröschel, M. (2012). Iron deficiency disrupts axon maturation of the developing auditory nerve. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 32*(14), 5010-5015. doi:10.1523/JNEUROSCI.0526-12.2012

Lee, E.-H. (2012). Review of the Psychometric Evidence of the Perceived Stress Scale. *Asian Nursing Research, 6*(4), 121-127. doi:https://doi.org/10.1016/j.anr.2012.08.004

Legrand, D., & Mazurier, J. (2010). A critical review of the roles of host lactoferrin in immunity. *Biometals, 23*(3), 365-376. doi:10.1007/s10534-010-9297-1

Leithner, C., & Royl, G. (2014). The oxygen paradox of neurovascular coupling. *J Cereb Blood Flow Metab, 34*(1), 19-29. doi:10.1038/jcbfm.2013.181

Lenz, C., Frietsch, T., Fütterer, C., van Ackern, K., Kuschinsky, W., & Waschke, K. F. (2000). Influence of blood viscosity on blood flow in the forebrain but not hindbrain after carotid occlusion in rats. *Journal of Cerebral Blood Flow & Metabolism, 20*(6), 947-955.

Leonard, A., Chalmers, K., Collins, C., & Patterson, A. (2014). A study of the effects of latent iron deficiency on measures of cognition: a pilot randomised controlled trial of iron supplementation in young women. *Nutrients, 6*(6), 2419-2435.

Leonard, A. J., Chalmers, K. A., Collins, C. E., & Patterson, A. J. (2014). Comparison of two doses of elemental iron in the treatment of latent iron deficiency: efficacy, side effects and blinding capabilities. *Nutrients, 6*(4), 1394-1405. doi:10.3390/nu6041394

Lever-van Milligen, B. A., Vogelzangs, N., Smit, J. H., & Penninx, B. W. (2014). Hemoglobin levels in persons with depressive and/or anxiety disorders. *J Psychosom Res, 76*(4), 317-321. doi:10.1016/j.jpsychores.2014.01.004

Levine, M., Wang, Y., Padayatty, S.J., & Morrow, J. (2001). A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci USA*, *98*, 9842-9846

Lewis, S. M., Osei-Bimpong, A., & Bradshaw, A. (2004). Measurement of haemoglobin as a screening test in general practice. *J Med Screen, 11*(2), 103-105. doi:10.1258/096914104774061100

Li, X., Allen, R. P., Earley, C. J., Liu, H., Cruz, T. E., Edden, R. A. E., . . . van Zijl, P. C. M. (2016). Brain iron deficiency in idiopathic restless legs syndrome measured by quantitative magnetic susceptibility at 7 tesla. *Sleep medicine, 22*, 75-82. doi:10.1016/j.sleep.2016.05.001

Li, Y., Kim, J., Buckett, P. D., Bohlke, M., Maher, T. J., & Wessling-Resnick, M. (2011). Severe postnatal iron deficiency alters emotional behavior and dopamine levels in the prefrontal cortex of young male rats. *J Nutr, 141*(12), 2133-2138. doi:10.3945/jn.111.145946

Li, Z., Li, B., Song, X., & Zhang, D. (2017). Dietary zinc and iron intake and risk of depression: A meta-analysis. *Psychiatry Research, 251*, 41-47. doi:https://doi.org/10.1016/j.psychres.2017.02.006

Lim, I. A. L., Faria, A. V., Li, X., Hsu, J. T. C., Airan, R. D., Mori, S., & van Zijl, P. C. M. (2013). Human brain atlas for automated region of interest selection in quantitative susceptibility mapping: application to determine iron content in deep gray matter structures. *NeuroImage, 82*, 449-469. doi:10.1016/j.neuroimage.2013.05.127

Lind, T., Lönnerdal, B., Stenlund, H., Gamayanti, I. L., Ismail, D., Seswandhana, R., & Persson, L.-Å. (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *The American journal of clinical nutrition, 80*(3), 729-736. doi:10.1093/ajcn/80.3.729

Lippi, G., Lima-Oliveira, G., Salvagno, G. L., Montagnana, M., Gelati, M., Picheth, G., . . . Guidi, G. C. (2010). Influence of a light meal on routine haematological tests. *Blood Transfusion, 8*(2), 94.

Lomagno, K. A., Hu, F., Riddell, L. J., Booth, A. O., Szymlek-Gay, E. A., Nowson, C. A., & Byrne, L. K. (2014). Increasing iron and zinc in pre-menopausal women and its effects on mood and cognition: a systematic review. *Nutrients, 6*(11), 5117-5141. doi:10.3390/nu6115117

Long, P., Wan, G., Roberts, M. T., & Corfas, G. (2018). Myelin development, plasticity, and pathology in the auditory system. *Developmental neurobiology, 78*(2), 80-92. doi:10.1002/dneu.22538

Lönnerdal, B. (2017). Development of iron homeostasis in infants and young children. *The American journal of clinical nutrition, 106*(Suppl 6), 1575S-1580S. doi:10.3945/ajcn.117.155820

Looker, A. C., Dallman, P. R., Carroll, M. D., Gunter, E. W., & Johnson, C. L. (1997). Prevalence of iron deficiency in the United States. *JAMA, 277*(12), 973-976. doi:10.1001/jama.1997.03540360041028

Lövdén, M., Fratiglioni, L., Glymour, M. M., Lindenberger, U., & Tucker-Drob, E. M. (2020). Education and Cognitive Functioning Across the Life Span. *Psychological Science in the Public Interest, 21*(1), 6-41. doi:10.1177/1529100620920576

Low, M., Farrell, A., Biggs, B.-A., & Pasricha, S.-R. (2013). Effects of daily iron supplementation in primary-school-aged children: systematic review and meta-analysis of randomized controlled trials. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 185*(17), E791-E802. doi:10.1503/cmaj.130628

Low, M. S., Speedy, J., Styles, C. E., De-Regil, L. M., & Pasricha, S. R. (2016). Daily iron supplementation for improving anaemia, iron status and health in menstruating women. *Cochrane Database Syst Rev, 4*, Cd009747. doi:10.1002/14651858.CD009747.pub2

Lozoff, B. (2011). Early iron deficiency has brain and behavior effects consistent with dopaminergic dysfunction. *The Journal of nutrition, 141*(4), 740S-746S. doi:10.3945/jn.110.131169

Lozoff, B., Beard, J., Connor, J., Barbara, F., Georgieff, M., & Schallert, T. (2006). Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutrition reviews, 64*(5 Pt 2), S34-S91. doi:10.1301/nr.2006.may.s34-s43

Lozoff, B., Brittenham, G., Viteri, F., & Urrutia, J. (1982). Behavioral abnormalities in infants with iron deficiency anemia.

Lozoff, B., Castillo, M., Clark, K. M., & Smith, J. B. (2012). Iron-Fortified vs Low-Iron Infant Formula: Developmental Outcome at 10 Years. *Archives of pediatrics & adolescent medicine, 166*(3), 208-215. doi:10.1001/archpediatrics.2011.197

Lozoff, B., Castillo, M., Clark, K. M., Smith, J. B., & Sturza, J. (2014). Iron Supplementation in Infancy Contributes to More Adaptive Behavior at 10 Years of Age. *The Journal of nutrition, 144*(6), 838-845. doi:10.3945/jn.113.182048

Lozoff, B., De Andraca, I., Castillo, M., Smith, J. B., Walter, T., & Pino, P. (2003). Behavioral and Developmental Effects of Preventing Iron-Deficiency Anemia in Healthy Full-Term Infants. *Pediatrics, 112*(4), 846-854. Retrieved from https://pediatrics.aappublications.org/content/pediatrics/112/4/846.full.pdf

Lozoff, B., & Georgieff, M. K. (2006). Iron deficiency and brain development. *Semin Pediatr Neurol, 13*(3), 158-165. doi:10.1016/j.spen.2006.08.004

Lozoff, B., Jimenez, E., Hagen, J., Mollen, E., & Wolf, A. W. (2000). Poorer Behavioral and Developmental Outcome More Than 10 Years After Treatment for Iron Deficiency in Infancy. *Pediatrics, 105*(4), e51. doi:10.1542/peds.105.4.e51

Lozoff, B., Jimenez, E., & Smith, J. B. (2006). Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. *Archives of pediatrics & adolescent medicine, 160*(11), 1108-1113. doi:10.1001/archpedi.160.11.1108

Lozoff, B., Jimenez, E., & Wolf, A. W. (1991). Long-term developmental outcome of infants with iron deficiency. *N Engl J Med, 325*(10), 687-694. doi:10.1056/nejm199109053251004

Lozoff, B., Smith, J. B., Kaciroti, N., Clark, K. M., Guevara, S., & Jimenez, E. (2013). Functional Significance of Early-Life Iron Deficiency: Outcomes at 25 Years. *The Journal of Pediatrics, 163*(5), 1260-1266. doi:https://doi.org/10.1016/j.jpeds.2013.05.015

Lua, P. L., & Wan Putri Elena, W. D. (2012). The impact of nutrition education interventions on the dietary habits of college students in developed nations: a brief review. *The Malaysian journal of medical sciences : MJMS, 19*(1), 4-14. Retrieved from https://pubmed.ncbi.nlm.nih.gov/22977369

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436500/

Lukes, A. S., Baker, J., Eder, S., & Adomako, T. L. (2012). Daily menstrual blood loss and quality of life in women with heavy menstrual bleeding. *Womens Health (Lond), 8*(5), 503-511. doi:10.2217/whe.12.36

Lukowski, A. F., Koss, M., Burden, M. J., Jonides, J., Nelson, C. A., Kaciroti, N., . . . Lozoff, B. (2010). Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. *Nutritional Neuroscience, 13*(2), 54-70. doi:10.1179/147683010X12611460763689

Lundgren-Nilsson, Å., Dencker, A., Jakobsson, S., Taft, C., & Tennant, A. (2014). Construct Validity of the Swedish Version of the Revised Piper Fatigue Scale in an Oncology

Sample—A Rasch Analysis. *Value in Health, 17*(4), 360-363. doi:https://doi.org/10.1016/j.jval.2014.02.010

Lykkesfeldt, J. (2002). Measurement of ascorbic acid and dehydroascorbic acid in biological samples. *Curr Protoc Toxicol, Chapter 7*, Unit 7.6.1-15. doi:10.1002/0471140856.tx0706s12

Ma, L., Xu, Y., Wang, G., & Li, R. (2019). What do we know about sex differences in depression: A review of animal models and potential mechanisms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 89*, 48-56. doi:https://doi.org/10.1016/j.pnpbp.2018.08.026

Mackler, B., Person, R., Miller, L. R., Inamdar, A. R., & Finch, C. A. (1978). Iron deficiency in the rat: biochemical studies of brain metabolism. *Pediatr Res, 12*(3), 217-220. doi:10.1203/00006450-197803000-00011

Madrid-Valero, J. J., Martínez-Selva, J. M., Ribeiro do Couto, B., Sánchez-Romera, J. F., & Ordoñana, J. R. (2017). Age and gender effects on the prevalence of poor sleep quality in the adult population. *Gaceta Sanitaria, 31*(1), 18-22. doi:https://doi.org/10.1016/j.gaceta.2016.05.013

Maheshwari, G., & Shaukat, F. (2019). Impact of Poor Sleep Quality on the Academic Performance of Medical Students. *Cureus, 11*(4), e4357. doi:10.7759/cureus.4357

Mairbäurl, H. (2013). Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Front Physiol, 4*, 332-332. doi:10.3389/fphys.2013.00332

Manji, H., Kato, T., Di Prospero, N. A., Ness, S., Beal, M. F., Krams, M., & Chen, G. (2012). Impaired mitochondrial function in psychiatric disorders. *Nat Rev Neurosci, 13*(5), 293-307. doi:10.1038/nrn3229

Marino, M., Li, Y., Rueschman, M. N., Winkelman, J. W., Ellenbogen, J. M., Solet, J. M., . . . Buxton, O. M. (2013). Measuring sleep: accuracy, sensitivity, and specificity of wrist actigraphy compared to polysomnography. *Sleep, 36*(11), 1747-1755. doi:10.5665/sleep.3142

Marques, D. R., Meia-Via, A. M. S., da Silva, C. F., & Gomes, A. A. (2017). Associations between sleep quality and domains of quality of life in a non-clinical sample: results from higher education students. *Sleep Health: Journal of the National Sleep Foundation, 3*(5), 348-356. doi:10.1016/j.sleh.2017.07.004

Mars, B., Heron, J., Kessler, D., Davies, N. M., Martin, R. M., Thomas, K. H., & Gunnell, D. (2017). Influences on antidepressant prescribing trends in the UK: 1995-2011. *Soc Psychiatry Psychiatr Epidemiol, 52*(2), 193-200. doi:10.1007/s00127-016-1306-4

Martin, J. L., & Hakim, A. D. (2011). Wrist actigraphy. *Chest, 139*(6), 1514-1527. doi:10.1378/chest.10-1872

Masini, A., Salvioli, G., Cremonesi, P., Botti, B., Gallesi, D., & Ceccarelli, D. (1994). Dietary iron deficiency in the rat. I. Abnormalities in energy metabolism of the hepatic tissue. *Biochimica et biophysica acta, 1188*(1-2), 46-52. doi:10.1016/0005-2728(94)90020-5

Masse, E., Salvail, H., Desnoyers, G., & Arguin, M. (2007). Small RNAs controlling iron metabolism. *Curr Opin Microbiol, 10*(2), 140-145. doi:10.1016/j.mib.2007.03.013

Mast, A. E., Blinder, M. A., Gronowski, A. M., Chumley, C., & Scott, M. G. (1998). Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clinical Chemistry, 44*(1), 45-51. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-0031972806&partnerID=40&md5=9eda62ab3c1e93cef0bd4b23d420674b

Matta, J., Czernichow, S., Kesse-Guyot, E., Hoertel, N., Limosin, F., Goldberg, M., . . . Lemogne, C. (2018). Depressive Symptoms and Vegetarian Diets: Results from the Constances Cohort. *Nutrients, 10*(11), 1695. doi:10.3390/nu10111695

Mayer, E. A. (2000a). The neurobiology of stress and gastrointestinal disease. *Gut, 47*(6), 861-869. doi:10.1136/gut.47.6.861

Mayer, E. A. (2000b). Psychological stress and colitis. *Gut, 46*(5), 595-596. doi:10.1136/gut.46.5.595

Mayer, E. A., Tillisch, K., & Gupta, A. (2015). Gut/brain axis and the microbiota. *J Clin Invest, 125*(3), 926-938. doi:10.1172/JCI76304

McArthur, J. O., Petocz, P., Caterson, I. D., & Samman, S. (2012). A randomized controlled trial in young women of the effects of consuming pork meat or iron supplements on nutritional status and feeling of well-being. *J Am Coll Nutr, 31*(3), 175-184. doi:10.1080/07315724.2012.10720025

McCabe, D. P., Roediger, H. L., McDaniel, M. A., Balota, D. A., & Hambrick, D. Z. (2010). The relationship between working memory capacity and executive functioning: evidence for a common executive attention construct. *Neuropsychology, 24*(2), 222-243. doi:10.1037/a0017619

McClung, J. P., Gaffney-Stomberg, E., & Lee, J. J. (2014). Female athletes: A population at risk of vitamin and mineral deficiencies affecting health and performance. *Journal of Trace Elements in Medicine and Biology, 28*(4), 388-392. doi:https://doi.org/10.1016/j.jtemb.2014.06.022

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *The American journal of clinical nutrition, 90*(1), 124-131. doi:10.3945/ajcn.2009.27774

McNair, D. M. (1992). Profile of mood states. *Educational and Industrial Testing Service*.

Meeusen, R., Watson, P., Hasegawa, H., Roelands, B., & Piacentini, M. F. (2006). Central Fatigue. *Sports Medicine, 36*(10), 881-909. doi:10.2165/00007256-200636100-00006

Meiergerd, S. M., Patterson, T. A., & Schenk, J. O. (1993). D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. *J Neurochem, 61*(2), 764-767. doi:10.1111/j.1471-4159.1993.tb02185.x

Mesias, M., Seiquer, I., & Navarro, M. (2013). Iron Nutrition in Adolescence. *Critical reviews in food science and nutrition, 53*, 1226-1237. doi:10.1080/10408398.2011.564333

Mila, M., Cecilia, A., Gracia, A., Antonio, M., Oscar, T., & Patricio, P. (2013). Evaluation of oral iron supplementation in pediatric maintenance insomnia. *Sleep medicine, 14*, e207-e208. doi:https://doi.org/10.1016/j.sleep.2013.11.496

Milman, N. (2011). Postpartum anemia I: definition, prevalence, causes, and consequences. *Ann Hematol, 90*(11), 1247-1253. doi:10.1007/s00277-011-1279-z

Milman, N., Jonsson, L., Dyre, P., Pedersen, P. L., & Larsen, L. G. (2014). Ferrous bisglycinate 25 mg iron is as effective as ferrous sulfate 50 mg iron in the prophylaxis of iron deficiency and anemia during pregnancy in a randomized trial. *J Perinat Med, 42*(2), 197-206. doi:10.1515/jpm-2013-0153

Milman, N., Taylor, C. L., Merkel, J., & Brannon, P. M. (2017). Iron status in pregnant women and women of reproductive age in Europe. *The American journal of clinical nutrition, 106*(Suppl 6), 1655s-1662s. doi:10.3945/ajcn.117.156000

Milman, N. T. (2019). Dietary Iron Intake in Women of Reproductive Age in Europe: A Review of 49 Studies from 29 Countries in the Period 1993–2015. *Journal of nutrition and metabolism, 2019*.

Minati, L., Visani, E., Dowell, N. G., Medford, N., & Critchley, H. D. (2011). Variability comparison of simultaneous brain near-infrared spectroscopy and functional magnetic resonance imaging during visual stimulation. *Journal of medical engineering & technology, 35*(6-7), 370-376. doi:10.3109/03091902.2011.595533

Mittal, R. D., Pandey, A., Mittal, B., & Agarwal, K. N. (2003). Effect of latent iron deficiency on GABA and glutamate neuroreceptors in rat brain. *Indian journal of clinical biochemistry : IJCB, 18*(1), 111-116. doi:10.1007/BF02867677

Mizuno, S., Mihara, T., Miyaoka, T., Inagaki, T., & Horiguchi, J. (2005). CSF iron, ferritin and transferrin levels in restless legs syndrome. *J Sleep Res, 14*(1), 43-47. doi:10.1111/j.1365-2869.2004.00403.x

Moffatt, M. E., Longstaffe, S., Besant, J., & Dureski, C. (1994). Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. *J Pediatr, 125*(4), 527-534. doi:10.1016/s0022-3476(94)70003-6

Mohammed, E. A., Naugler, C., & Far, B. H. (2015). Chapter 32 - Emerging Business Intelligence Framework for a Clinical Laboratory Through Big Data Analytics. In Q. N. Tran & H. Arabnia (Eds.), *Emerging Trends in Computational Biology, Bioinformatics, and Systems Biology* (pp. 577-602). Boston: Morgan Kaufmann.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ (Clinical research ed.), 339*, b2535. doi:10.1136/bmj.b2535

Moisan, F., Kab, S., Mohamed, F., Canonico, M., Le Guern, M., Quintin, C., . . . Elbaz, A. (2016). Parkinson disease male-to-female ratios increase with age: French nationwide study and meta-analysis. *Journal of Neurology, Neurosurgery & Psychiatry, 87*(9), 952-957. doi:10.1136/jnnp-2015-312283

Monti, J. M., & Jantos, H. (2008). The roles of dopamine and serotonin, and of their receptors, in regulating sleep and waking. *Prog Brain Res, 172*, 625-646. doi:10.1016/s0079-6123(08)00929-1

Monti, J. M., & Monti, D. (2007). The involvement of dopamine in the modulation of sleep and waking. *Sleep medicine reviews, 11*(2), 113-133.

More, S., Shivkumar, V. B., Gangane, N., & Shende, S. (2013). Effects of iron deficiency on cognitive function in school going adolescent females in rural area of central India. *Anemia, 2013*, 819136-819136. doi:10.1155/2013/819136

Moreno Chulilla, J. A., Romero Colás, M. S., & Gutiérrez Martín, M. (2009). Classification of anemia for gastroenterologists. *World journal of gastroenterology, 15*(37), 4627-4637. doi:10.3748/wjg.15.4627

Moreno-Fernandez, J., Ochoa, J. J., Latunde-Dada, G. O., & Diaz-Castro, J. (2019). Iron Deficiency and Iron Homeostasis in Low Birth Weight Preterm Infants: A Systematic Review. *Nutrients, 11*(5), 1090. doi:10.3390/nu11051090

Moretti, D., Goede, J. S., Zeder, C., Jiskra, M., Chatzinakou, V., Tjalsma, H., . . . Zimmermann, M. B. (2015). Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood, 126*(17), 1981-1989. doi:10.1182/blood-2015-05-642223

Morse, A. C., Beard, J. L., Azar, M. R., & Jones, B. C. (1999). Sex and Genetics are Important Cofactors in Assessing the Impact of Iron Deficiency on the Developing Mouse Brain. *Nutr Neurosci, 2*(5), 323-335. doi:10.1080/1028415x.1999.11747287

Moschonis, G., Papandreou, D., Mavrogianni, C., Giannopoulou, A., Damianidi, L., Malindretos, P., . . . Manios, Y. (2013). Association of iron depletion with menstruation and dietary intake indices in pubertal girls: the healthy growth study. *BioMed research international, 2013*, 423263-423263. doi:10.1155/2013/423263

Mousa, S. O., Higazi, A. M., Saleh, S. M., & Ali, H. A. (2016). Cognitive Function and School Achievement in Adolescent Egyptian Girls with Iron Deficiency and Iron Deficiency Anaemia. *Ment Health Fam Med, 12*, 289-294.

Mudd, A. T., Fil, J. E., Knight, L. C., Lam, F., Liang, Z.-P., & Dilger, R. N. (2018). Early-Life Iron Deficiency Reduces Brain Iron Content and Alters Brain Tissue Composition Despite Iron Repletion: A Neuroimaging Assessment. *Nutrients, 10*(2), 135. doi:10.3390/nu10020135

Müller, M. M., Gruber, T., & Keil, A. (2000). Modulation of induced gamma band activity in the human EEG by attention and visual information processing. *International Journal of Psychophysiology, 38*(3), 283-299. doi:https://doi.org/10.1016/S0167-8760(00)00171-9

Mulligan, A. A., Luben, R. N., Bhaniani, A., Parry-Smith, D. J., O'Connor, L., Khawaja, A. P., . . . Khaw, K.-T. (2014). A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open, 4*(3), e004503. doi:10.1136/bmjopen-2013-004503

Munzer, T., & Felt, B. (2017). *The role of iron in pediatric restless legs syndrome and periodic limb movements in sleep.* Paper presented at the Seminars in neurology.

Murat, S., Ali, U., Serdal, K., Süleyman, D., İlknur, P., Mehmet, S., . . . Tunahan, U. (2015). Assessment of subjective sleep quality in iron deficiency anaemia. *African health sciences, 15*(2), 621-627. doi:10.4314/ahs.v15i2.40

Murray-Kolb, L. E., & Beard, J. L. (2007). Iron treatment normalizes cognitive functioning in young women. *The American journal of clinical nutrition, 85*(3), 778-787. doi:10.1093/ajcn/85.3.778

Murray-Kolb, L. E., Khatry, S. K., Katz, J., Schaefer, B. A., Cole, P. M., LeClerq, S. C., . . . Christian, P. (2012). Preschool Micronutrient Supplementation Effects on Intellectual and Motor Function in School-aged Nepalese Children. *Archives of pediatrics & adolescent medicine, 166*(5), 404-410. doi:10.1001/archpediatrics.2012.37

Murray-Kolb, L. E., Wenger, M. J., Scott, S. P., Rhoten, S. E., Lung'aho, M. G., & Haas, J. D. (2017). Consumption of Iron-Biofortified Beans Positively Affects Cognitive Performance in 18- to 27-Year-Old Rwandan Female College Students in an 18-Week Randomized Controlled Efficacy Trial. *J Nutr, 147*(11), 2109-2117. doi:10.3945/jn.117.255356

Naef, M., Müller, U., Linssen, A., Clark, L., Robbins, T. W., & Eisenegger, C. (2017). Effects of dopamine D2/D3 receptor antagonism on human planning and spatial working memory. *Translational Psychiatry, 7*(4), e1107-e1107. doi:10.1038/tp.2017.56

Naghedi-Baghdar, H., Nazari, S. M., Taghipour, A., Nematy, M., Shokri, S., Mehri, M. R., . . . Javan, R. (2018). Effect of diet on blood viscosity in healthy humans: a systematic review. *Electron Physician, 10*(3), 6563-6570. doi:10.19082/6563

Nairz, M., Haschka, D., Demetz, E., & Weiss, G. (2014). Iron at the interface of immunity and infection. *Front Pharmacol, 5*, 152-152. doi:10.3389/fphar.2014.00152

Nairz, M., Theurl, I., Wolf, D., & Weiss, G. (2016). Iron deficiency or anemia of inflammation? : Differential diagnosis and mechanisms of anemia of inflammation. *Wien Med Wochenschr, 166*(13-14), 411-423. doi:10.1007/s10354-016-0505-7

Nakamura, T., Naguro, I., & Ichijo, H. (2019). Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. *Biochim Biophys Acta Gen Subj, 1863*(9), 1398-1409. doi:10.1016/j.bbagen.2019.06.010

Namaste, S. M., Rohner, F., Huang, J., Bhushan, N. L., Flores-Ayala, R., Kupka, R., . . . Suchdev, P. S. (2017). Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *The American journal of clinical nutrition, 106*(Suppl 1), 359S-371S. doi:10.3945/ajcn.116.141762

Name, J. J., Vasconcelos, A. R., & Valzachi Rocha Maluf, M. C. (2018). Iron Bisglycinate Chelate and Polymaltose Iron for the Treatment of Iron Deficiency Anemia: A Pilot Randomized Trial. *Current pediatric reviews, 14*(4), 261-268. doi:10.2174/1573396314666181002170040

Nave, K. A., & Werner, H. B. (2014). Myelination of the nervous system: mechanisms and functions. *Annu Rev Cell Dev Biol, 30*, 503-533. doi:10.1146/annurev-cellbio-100913-013101

Ndayisaba, A., Kaindlstorfer, C., & Wenning, G. K. (2019). Iron in Neurodegeneration - Cause or Consequence? *Frontiers in neuroscience, 13*, 180-180. doi:10.3389/fnins.2019.00180

Nehring, S. M., Goyal, A., Bansal, P., & Patel, B. C. (2020). C Reactive Protein (CRP). In *StatPearls*. Treasure Island (FL): StatPearls Publishing

Nelson, C., Erikson, K., Pinero, D. J., & Beard, J. L. (1997). In vivo dopamine metabolism is altered in iron-deficient anemic rats. *J Nutr, 127*(12), 2282-2288. doi:10.1093/jn/127.12.2282

Nelson, M. C., Taylor, K., & Vella, C. A. (2019). Comparison of Self-Reported and Objectively Measured Sedentary Behavior and Physical Activity in Undergraduate Students. *Measurement in Physical Education and Exercise Science, 23*(3), 237-248. doi:10.1080/1091367X.2019.1610765

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., & Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest, 113*(9), 1271-1276. doi:10.1172/jci20945

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., . . . Kaplan, J. (2004). Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. *Science, 306*(5704), 2090-2093. doi:10.1126/science.1104742

Nickel, M., & Gu, C. (2018). Regulation of Central Nervous System Myelination in Higher Brain Functions. *Neural Plast, 2018*, 6436453. doi:10.1155/2018/6436453

Nicolson, G. L. (2014). Mitochondrial Dysfunction and Chronic Disease: Treatment With Natural Supplements. *Integrative medicine (Encinitas, Calif.), 13*(4), 35-43. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26770107

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4566449/

Nieoullon, A. (2002). Dopamine and the regulation of cognition and attention. *Prog Neurobiol, 67*(1), 53-83. doi:10.1016/s0301-0082(02)00011-4

Nomura, Y., John, R. M., Janssen, A. B., Davey, C., Finik, J., Buthmann, J., . . . Lambertini, L. (2017). Neurodevelopmental consequences in offspring of mothers with preeclampsia during pregnancy: underlying biological mechanism via imprinting genes. *Archives of Gynecology and Obstetrics, 295*(6), 1319-1329. doi:10.1007/s00404-017-4347-3

Northrop-Clewes, C. A. (2008). Interpreting indicators of iron status during an acute phase response--lessons from malaria and human immunodeficiency virus. *Ann Clin Biochem, 45*(Pt 1), 18-32. doi:10.1258/acb.2007.007167

Northrop-Clewes, C. A., & Thurnham, D. I. (2013). Biomarkers for the differentiation of anemia and their clinical usefulness. *Journal of blood medicine, 4*, 11-22. doi:10.2147/JBM.S29212

Nyberg, L., Karalija, N., Salami, A., Andersson, M., Wåhlin, A., Kaboovand, N., . . . Bäckman, L. (2016). Dopamine D2 receptor availability is linked to hippocampal–caudate functional connectivity and episodic memory. *Proceedings of the National Academy of Sciences, 113*(28), 7918-7923. doi:10.1073/pnas.1606309113

Oberman, L., & Pascual-Leone, A. (2013). Changes in plasticity across the lifespan: cause of disease and target for intervention. *Prog Brain Res, 207*, 91-120. doi:10.1016/b978-0-444-63327-9.00016-3

Ohlendieck, K. (2013). Proteomic identification of biomarkers of skeletal muscle disorders. *Biomark Med, 7*(1), 169-186. doi:10.2217/bmm.12.96

Olivares, M., Pizarro, F., Pineda, O., Name, J. J., Hertrampf, E., & Walter, T. s. (1997). Milk Inhibits and Ascorbic Acid Favors Ferrous Bis-Glycine Chelate Bioavailability in Humans. *The Journal of nutrition, 127*(7), 1407-1411. doi:10.1093/jn/127.7.1407

Opie, R. S., Itsiopoulos, C., Parletta, N., Sanchez-Villegas, A., Akbaraly, T. N., Ruusunen, A., & Jacka, F. N. (2017). Dietary recommendations for the prevention of depression. *Nutritional Neuroscience, 20*(3), 161-171. doi:10.1179/1476830515Y.0000000043

Opie, R. S., O'Neil, A., Jacka, F. N., Pizzinga, J., & Itsiopoulos, C. (2018). A modified Mediterranean dietary intervention for adults with major depression: Dietary protocol and feasibility data from the SMILES trial. *Nutr Neurosci, 21*(7), 487-501. doi:10.1080/1028415x.2017.1312841

Organization, W. H. (2011a). *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. Retrieved from

Organization, W. H. (2011b). *Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations*. Retrieved from

Ortiz, E., Pasquini, J. M., Thompson, K., Felt, B., Butkus, G., Beard, J., & Connor, J. R. (2004). Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. *Journal of Neuroscience Research, 77*(5), 681-689. doi:10.1002/jnr.20207

Ortiz, R., Toblli, J. E., Romero, J. D., Monterrosa, B., Frer, C., Macagno, E., & Breymann, C. (2011). Efficacy and safety of oral iron(III) polymaltose complex versus ferrous sulfate in pregnant women with iron-deficiency anemia: a multicenter, randomized, controlled study. *J Matern Fetal Neonatal Med, 24*(11), 1347-1352. doi:10.3109/14767058.2011.599080

Owen, L., & Sunram-Lea, S. I. (2011). Metabolic Agents that Enhance ATP can Improve Cognitive Functioning: A Review of the Evidence for Glucose, Oxygen, Pyruvate, Creatine, and l-Carnitine. *Nutrients, 3*(8), 735-755. Retrieved from https://www.mdpi.com/2072-6643/3/8/735

Pamuk, G. E., Uyanik, M. S., Top, M. S., Tapan, U., Ak, R., & Uyanik, V. (2015). Gastrointestinal symptoms are closely associated with depression in iron deficiency anemia: a comparative study. *Annals of Saudi medicine, 35*(1), 31-35. doi:10.5144/0256-4947.2015.31

Paradkar, P. N., De Domenico, I., Durchfort, N., Zohn, I., Kaplan, J., & Ward, D. M. (2008). Iron depletion limits intracellular bacterial growth in macrophages. *Blood, 112*(3), 866-874. doi:10.1182/blood-2007-12-126854

Parazzini, C., Baldoli, C., Scotti, G., & Triulzi, F. (2002). Terminal zones of myelination: MR evaluation of children aged 20-40 months. *AJNR Am J Neuroradiol, 23*(10), 1669-1673.

Pasricha, S.-R., Low, M., Thompson, J., Farrell, A., & De-Regil, L.-M. (2014). Iron Supplementation Benefits Physical Performance in Women of Reproductive Age: A Systematic Review and Meta-Analysis. *The Journal of nutrition, 144*(6), 906-914. doi:10.3945/jn.113.189589

Pasricha, S.-R. S., Flecknoe-Brown, S. C., Allen, K. J., Gibson, P. R., McMahon, L. P., Olynyk, J. K., . . . Robinson, K. L. (2010). Diagnosis and management of iron deficiency anaemia: a clinical update. *Medical Journal of Australia, 193*(9), 525-532. doi:10.5694/j.1326-5377.2010.tb04038.x

Pasricha, S. R., Hayes, E., Kalumba, K., & Biggs, B. A. (2013). Effect of daily iron supplementation on health in children aged 4-23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet Glob Health, 1*(2), e77-e86. doi:10.1016/s2214-109x(13)70046-9

Patterson, A. J., Brown, W. J., Powers, J. R., & Roberts, D. C. K. (2000). Iron deficiency, general health and fatigue: Results from the Australian Longitudinal Study on Women's Health. *Quality of Life Research, 9*(5), 491-497. doi:10.1023/A:1008978114650

Patterson, A. J., Brown, W. J., & Roberts, D. C. (2001). Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. *J Am Coll Nutr, 20*(4), 337-342. doi:10.1080/07315724.2001.10719054

Paul, B. T., Manz, D. H., Torti, F. M., & Torti, S. V. (2017). Mitochondria and Iron: current questions. *Expert Rev Hematol, 10*(1), 65-79. doi:10.1080/17474086.2016.1268047

Peeling, P. (2010). Exercise as a mediator of hepcidin activity in athletes. *Eur J Appl Physiol, 110*(5), 877-883. doi:10.1007/s00421-010-1594-4

Peeling, P., Sim, M., Badenhorst, C. E., Dawson, B., Govus, A. D., Abbiss, C. R., . . . Trinder, D. (2014). Iron status and the acute post-exercise hepcidin response in athletes. *PLoS One, 9*(3), e93002-e93002. doi:10.1371/journal.pone.0093002

Peirano, P. D., Algarín, C. R., Chamorro, R. A., Reyes, S. C., Durán, S. A., Garrido, M. I., & Lozoff, B. (2010). Sleep alterations and iron deficiency anemia in infancy. *Sleep medicine, 11*(7), 637-642. doi:10.1016/j.sleep.2010.03.014

Pellicer, A., & Bravo, M. d. C. (2011). Near-infrared spectroscopy: A methodology-focused review. *Seminars in Fetal and Neonatal Medicine, 16*(1), 42-49. doi:https://doi.org/10.1016/j.siny.2010.05.003

Percy, L., Mansour, D., & Fraser, I. (2017). Iron deficiency and iron deficiency anaemia in women. *Best Pract Res Clin Obstet Gynaecol, 40*, 55-67. doi:10.1016/j.bpobgyn.2016.09.007

Pereira, A. A., & Sarnak, M. J. (2003). Anemia as a risk factor for cardiovascular disease. *Kidney Int Suppl*(87), S32-39. doi:10.1046/j.1523-1755.64.s87.6.x

Perlman, S. B., Luna, B., Hein, T. C., & Huppert, T. J. (2014). fNIRS evidence of prefrontal regulation of frustration in early childhood. *NeuroImage, 85 Pt 1*(0 1), 326-334. doi:10.1016/j.neuroimage.2013.04.057

Petrie, K. J., & Rief, W. (2019). Psychobiological Mechanisms of Placebo and Nocebo Effects: Pathways to Improve Treatments and Reduce Side Effects. *Annual Review of Psychology, 70*(1), 599-625. doi:10.1146/annurev-psych-010418-102907

Pfeiffer, C. M., & Looker, A. C. (2017). Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *The American journal of clinical nutrition, 106*(Suppl 6), 1606S-1614S. doi:10.3945/ajcn.117.155887

PHE. (2016). Government Dietary Recommendations: Government Recommendations for Energy and Nutrients for Males and Females Aged 1-18 Years and 19+ Years. In: PHE London.

PHE (2014). National Diet and Nutrition Survey. Results from Years 1–4 (Combined) of the Rolling Programme (2008/2009–2011/12). In: PHE London.

Phillips, A. A., Chan, F. H., Zheng, M. M. Z., Krassioukov, A. V., & Ainslie, P. N. (2016). Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. *J Cereb Blood Flow Metab, 36*(4), 647-664. doi:10.1177/0271678X15617954

Pineda, O., & Ashmead, H. D. (2001). Effectiveness of treatment of iron-deficiency anemia in infants and young children with ferrous bis-glycinate chelate. *Nutrition, 17*(5), 381-384. doi:10.1016/s0899-9007(01)00519-6

Pineda, O., Wayne Ashmead, H. D., Perez, J. M., & Lemus, C. P. (1994). Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. *Journal of Applied Nutrition, 46*(1), 2-13.

Pino, J. M. V., da Luz, M. H. M., Antunes, H. K. M., Giampá, S. Q. d. C., Martins, V. R., & Lee, K. S. (2017). Iron-Restricted Diet Affects Brain Ferritin Levels, Dopamine Metabolism and Cellular Prion Protein in a Region-Specific Manner. *Frontiers in molecular neuroscience, 10*, 145-145. doi:10.3389/fnmol.2017.00145

Piper, B. F., Dibble, S. L., Dodd, M. J., Weiss, M. C., Slaughter, R. E., & Paul, S. M. (1998). The Revised Piper Fatigue Scale: Psychometric evaluation in women with breast cancer. *Oncology Nursing Forum, 25*, 677-684.

Pittman, R. N. (2011). *Regulation of tissue oxygenation.* Paper presented at the Colloquium series on integrated systems physiology: from molecule to function.

Pitzer, M. (2019). The development of monoaminergic neurotransmitter systems in childhood and adolescence. *International Journal of Developmental Neuroscience, 74*, 49-55. doi:https://doi.org/10.1016/j.ijdevneu.2019.02.002

Pletzer, B., Harris, T.-A., Scheuringer, A., & Hidalgo-Lopez, E. (2019). The cycling brain: menstrual cycle related fluctuations in hippocampal and fronto-striatal activation and connectivity during cognitive tasks. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 44*(11), 1867-1875. doi:10.1038/s41386-019-0435-3

Pompano, L. M., & Haas, J. D. (2017). Efficacy of iron supplementation may be misinterpreted using conventional measures of iron status in iron-depleted, nonanemic women undergoing aerobic exercise training. *The American journal of clinical nutrition, 106*(6), 1529-1538. doi:10.3945/ajcn.117.152777

Pongcharoen, T., DiGirolamo, A. M., Ramakrishnan, U., Winichagoon, P., Flores, R., & Martorell, R. (2011). Long-term effects of iron and zinc supplementation during infancy on cognitive function at 9 y of age in northeast Thai children: a follow-up study1–3. *The American journal of clinical nutrition, 93*(3), 636-643. doi:10.3945/ajcn.110.002220

Ponorac, N., Popović, M., karaba jakovljevic, D., Bajić, Z., Scanlan, A., Stojanović, E., & Radovanović, D. (2019). Professional Female Athletes Are at a Heightened Risk of Iron-Deficient Erythropoiesis Compared With Nonathletes. *Int J Sport Nutr Exerc Metab*, 1-6. doi:10.1123/ijsnem.2019-0193

Porto, G., & De Sousa, M. (2007). Iron overload and immunity. *World journal of gastroenterology, 13*(35), 4707-4715. doi:10.3748/wjg.v13.i35.4707

Power, J. D., & Schlaggar, B. L. (2017). Neural plasticity across the lifespan. *Wiley interdisciplinary reviews. Developmental biology, 6*(1), 10.1002/wdev.1216. doi:10.1002/wdev.216

Pra, D., Franke, S. I., Henriques, J. A., & Fenech, M. (2012). Iron and genome stability: an update. *Mutat Res, 733*(1-2), 92-99. doi:10.1016/j.mrfmmm.2012.02.001

Prentice, A. M., Mendoza, Y. A., Pereira, D., Cerami, C., Wegmuller, R., Constable, A., & Spieldenner, J. (2017). Dietary strategies for improving iron status: balancing safety and efficacy. *Nutrition reviews, 75*(1), 49-60. doi:10.1093/nutrit/nuw055

Price, K. L., Abernathy, B. E., Dobbs, J. C., & Gallaher, D. D. (2017). Iron Deficiency and Depression in Female State Fair Attendees. *The FASEB Journal, 31*(1_supplement), 298.296-298.296. doi:10.1096/fasebj.31.1_supplement.298.6

Prohan, M., Amani, R., Nematpour, S., Jomehzadeh, N., & Haghighizadeh, M. H. (2014). Total antioxidant capacity of diet and serum, dietary antioxidant vitamins intake, and

serum hs-CRP levels in relation to depression scales in university male students. *Redox Rep, 19*(3), 133-139. doi:10.1179/1351000214y.0000000085

Puig, S., Ramos-Alonso, L., Romero, A. M., & Martinez-Pastor, M. T. (2017). The elemental role of iron in DNA synthesis and repair. *Metallomics : integrated biometal science, 9*(11), 1483-1500. doi:10.1039/c7mt00116a

Puig, S., Vergara, S. V., & Thiele, D. J. (2008). Cooperation of two mRNA-binding proteins drives metabolic adaptation to iron deficiency. *Cell Metab, 7*(6), 555-564. doi:10.1016/j.cmet.2008.04.010

Pullar, J. M., Carr, A. C., Bozonet, S. M., & Vissers, M. C. M. (2018). High Vitamin C Status Is Associated with Elevated Mood in Male Tertiary Students. *Antioxidants (Basel, Switzerland), 7*(7), 91. doi:10.3390/antiox7070091

Pullar, T., Kumar, S., Tindall, H., & Feely, M. (1989). Time to stop counting the tablets? *Clinical Pharmacology & Therapeutics, 46*(2), 163-168.

Qin, T., Yan, M., Fu, Z., Song, Y., Lu, W., Fu, A. d., & Yin, P. (2019). Association between anemia and cognitive decline among Chinese middle-aged and elderly: evidence from the China health and retirement longitudinal study. *BMC Geriatrics, 19*(1), 305. doi:10.1186/s12877-019-1308-7

Quehl, R., Haines, J., Lewis, S. P., & Buchholz, A. C. (2017). Food and Mood: Diet Quality is Inversely Associated with Depressive Symptoms in Female University Students. *Canadian Journal of Dietetic Practice and Research, 78*(3), 124-128. doi:10.3148/cjdpr-2017-007

Radlowski, E. C., & Johnson, R. W. (2013). Perinatal iron deficiency and neurocognitive development. *Frontiers in human neuroscience, 7*, 585-585. doi:10.3389/fnhum.2013.00585

Rajagopal, A., Rao, A. U., Amigo, J., Tian, M., Upadhyay, S. K., Hall, C., . . . Hamza, I. (2008). Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. *Nature, 453*(7198), 1127-1131. doi:10.1038/nature06934

Rangan, A. M., Blight, G. D., & Binns, C. W. (1998). Iron status and non-specific symptoms of female students. *J Am Coll Nutr, 17*(4), 351-355. doi:10.1080/07315724.1998.10718774

Rao, R., & Georgieff, M. (2002). Perinatal aspects of iron metabolism. *Acta Paediatrica, 91*(s438), 124-129. doi:10.1111/j.1651-2227.2002.tb02917.x

Rao, R., Tkac, I., Schmidt, A. T., & Georgieff, M. K. (2011). Fetal and neonatal iron deficiency causes volume loss and alters the neurochemical profile of the adult rat hippocampus. *Nutritional Neuroscience, 14*(2), 59-65. doi:10.1179/1476830511Y.0000000001

Rao, R., Tkac, I., Townsend, E. L., Gruetter, R., & Georgieff, M. K. (2003). Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. *J Nutr, 133*(10), 3215-3221. doi:10.1093/jn/133.10.3215

Rao, T. S. S., Asha, M. R., Ramesh, B. N., & Rao, K. S. J. (2008). Understanding nutrition, depression and mental illnesses. *Indian journal of psychiatry, 50*(2), 77-82. doi:10.4103/0019-5545.42391

Rausa, M., Pagani, A., Nai, A., Campanella, A., Gilberti, M. E., Apostoli, P., . . . Silvestri, L. (2015). Bmp6 expression in murine liver non parenchymal cells: a mechanism to control their high iron exporter activity and protect hepatocytes from iron overload? *PLoS One, 10*(4), e0122696. doi:10.1371/journal.pone.0122696

Rebar, A. L., Stanton, R., Geard, D., Short, C., Duncan, M. J., & Vandelanotte, C. (2015). A meta-meta-analysis of the effect of physical activity on depression and anxiety in non-clinical adult populations. *Health Psychology Review, 9*(3), 366-378. doi:10.1080/17437199.2015.1022901

Reddy K, V., Shastry, S., Raturi, M., & Baliga B, P. (2020). Impact of Regular Whole-Blood Donation on Body Iron Stores. *Transfusion Medicine and Hemotherapy, 47*(1), 75-79. doi:10.1159/000499768

Reeve, B. B., Stover, A. M., Alfano, C. M., Smith, A. W., Ballard-Barbash, R., Bernstein, L., . . . Piper, B. F. (2012). The Piper Fatigue Scale-12 (PFS-12): psychometric findings and item reduction in a cohort of breast cancer survivors. *Breast cancer research and treatment, 136*(1), 9-20. doi:10.1007/s10549-012-2212-4

Rezaeian, A., Ghayour-Mobarhan, M., Mazloum, S. R., Yavari, M., & Jafari, S. A. (2014). Effects of iron supplementation twice a week on attention score and haematologic measures in female high school students. *Singapore Med J, 55*(11), 587-592. doi:10.11622/smedj.2014156

Ridsdale, L., Evans, A., Jerrett, W., Mandalia, S., Osler, K., & Vora, H. (1993). Patients with fatigue in general practice: a prospective study. *BMJ (Clinical research ed.), 307*(6896), 103-106.

Riggins, T., Miller, N. C., Bauer, P. J., Georgieff, M. K., & Nelson, C. A. (2009). Consequences of low neonatal iron status due to maternal diabetes mellitus on explicit memory performance in childhood. *Dev Neuropsychol, 34*(6), 762-779. doi:10.1080/87565640903265145

Ritchie, S. J., Bates, T. C., Der, G., Starr, J. M., & Deary, I. J. (2013). Education is associated with higher later life IQ scores, but not with faster cognitive processing speed. *Psychology and Aging, 28*(2), 515-521. doi:10.1037/a0030820

Roberts, K. M., & Fitzpatrick, P. F. (2013). Mechanisms of tryptophan and tyrosine hydroxylase. *IUBMB life, 65*(4), 350-357. doi:10.1002/iub.1144

Robitaille, L., & Hoffer, L. J. (2016). A simple method for plasma total vitamin C analysis suitable for routine clinical laboratory use. *Nutrition journal, 15*(1), 40. doi:10.1186/s12937-016-0158-9

Roncagliolo, M., Garrido, M., Walter, T., Peirano, P., & Lozoff, B. (1998). Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. *The American journal of clinical nutrition, 68*(3), 683-690. doi:10.1093/ajcn/68.3.683

Rubeor, A., Goojha, C., Manning, J., & White, J. (2018). Does Iron Supplementation Improve Performance in Iron-Deficient Nonanemic Athletes? *Sports health, 10*(5), 400-405. doi:10.1177/1941738118777488

Rupawala, M., Dehghani, H., Lucas, S. J. E., Tino, P., & Cruse, D. (2018). Shining a Light on Awareness: A Review of Functional Near-Infrared Spectroscopy for Prolonged Disorders of Consciousness. *Front Neurol, 9*, 350. doi:10.3389/fneur.2018.00350

Sachdev, H., Gera, T., & Nestel, P. (2005). Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public health nutrition, 8*(2), 117-132.

Scientific Advisory Committee on Nutrition. (2010) Iron and Health. TSO: London, UK.

Sadeghian, M. H., Keramati, M. R., Ayatollahi, H., Manavifar, L., Enaiati, H., & Mahmoudi, M. (2010). Serum immunoglobulins in patients with iron deficiency anemia. *Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion, 26*(2), 45-48. doi:10.1007/s12288-010-0025-3

Saha, L., Pandhi, P., Gopalan, S., Malhotra, S., & Saha, P. K. (2007). Comparison of efficacy, tolerability, and cost of iron polymaltose complex with ferrous sulphate in the treatment of iron deficiency anemia in pregnant women. *MedGenMed, 9*(1), 1.

Sakai, H., Murakami, K., Kobayashi, S., Suga, H., & Sasaki, S. (2017). Food-based diet quality score in relation to depressive symptoms in young and middle-aged Japanese women. *British Journal of Nutrition, 117*(12), 1674-1681. doi:10.1017/S0007114517001581

Salas-GÛmez, D., Fernandez-Gorgojo, M., Pozueta, A., Diaz-Ceballos, I., Lamarain, M., PÈrez, C. L., . . . S·nchez-Ju·n, P. (2020). Physical Activity Is Associated With Better Executive Function in University Students. *Frontiers in Human Neuroscience, 14*.

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiology of aging, 30*(4), 507-514. doi:10.1016/j.neurobiolaging.2008.09.023

Salthouse, T. A. (2019). Trajectories of normal cognitive aging. *Psychology and Aging, 34*(1), 17.

Sandstrom, B. (2001). Micronutrient interactions: effects on absorption and bioavailability. *Br J Nutr, 85 Suppl 2*, S181-185.

Sandstrom, B., Davidsson, L., Cederblad, A., & Lonnerdal, B. (1985). Oral iron, dietary ligands and zinc absorption. *J Nutr, 115*(3), 411-414. doi:10.1093/jn/115.3.411

Santiago, P. (2012). Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. *ScientificWorldJournal, 2012*, 846824. doi:10.1100/2012/846824

Sanvisens, N., Bano, M. C., Huang, M., & Puig, S. (2011). Regulation of ribonucleotide reductase in response to iron deficiency. *Mol Cell, 44*(5), 759-769. doi:10.1016/j.molcel.2011.09.021

Sarris, J., Logan, A. C., Akbaraly, T. N., Amminger, G. P., Balanza-Martinez, V., Freeman, M. P., . . . Jacka, F. N. (2015). Nutritional medicine as mainstream in psychiatry. *Lancet Psychiatry, 2*(3), 271-274. doi:10.1016/s2215-0366(14)00051-0

Saunders, A. V., Craig, W. J., Baines, S. K., & Posen, J. S. (2013). Iron and vegetarian diets. *Medical Journal of Australia, 199*(S4), S11-S16. doi:10.5694/mja11.11494

Sawada, T., Konomi, A., & Yokoi, K. (2014). Iron deficiency without anemia is associated with anger and fatigue in young Japanese women. *Biol Trace Elem Res, 159*(1-3), 22-31. doi:10.1007/s12011-014-9963-1

Scheeren, T. W., Schober, P., & Schwarte, L. A. (2012). Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications. *J Clin Monit Comput, 26*(4), 279-287. doi:10.1007/s10877-012-9348-y

Scheiber, I.F., Mercer, J.F.B., & Dringen, R. (2014). Metabolism and functions of copper in the brain. *Prog Neurobiol, 116*, 33-57

Schmidt, A. T., Alvarez, G. C., Grove, W. M., Rao, R., & Georgieff, M. K. (2012). Early iron deficiency enhances stimulus-response learning of adult rats in the context of competing spatial information. *Developmental cognitive neuroscience, 2*(1), 174-180. doi:10.1016/j.dcn.2011.07.014

Scholl, T. O. (2011). Maternal iron status: relation to fetal growth, length of gestation, and iron endowment of the neonate. *Nutrition reviews, 69 Suppl 1*, S23-29. doi:10.1111/j.1753-4887.2011.00429.x

Scott, S. P., De Souza, M. J., Koehler, K., & Murray-Kolb, L. E. (2017). Combined Iron Deficiency and Low Aerobic Fitness Doubly Burden Academic Performance among Women Attending University. *J Nutr, 147*(1), 104-109. doi:10.3945/jn.116.240192

Scott, S. P., & Murray-Kolb, L. E. (2016). Iron Status Is Associated with Performance on Executive Functioning Tasks in Nonanemic Young Women. *J Nutr, 146*(1), 30-37. doi:10.3945/jn.115.223586

Scott, S. P., Murray-Kolb, L. E., Wenger, M. J., Udipi, S. A., Ghugre, P. S., Boy, E., & Haas, J. D. (2018). Cognitive Performance in Indian School-Going Adolescents Is Positively Affected by Consumption of Iron-Biofortified Pearl Millet: A 6-Month Randomized

Controlled Efficacy Trial. *The Journal of nutrition, 148*(9), 1462-1471. doi:10.1093/jn/nxy113

Sekhar, D. L., Murray-Kolb, L. E., Kunselman, A. R., Weisman, C. S., & Paul, I. M. (2017). Association between menarche and iron deficiency in non-anemic young women. *PLoS One, 12*(5), e0177183-e0177183. doi:10.1371/journal.pone.0177183

Selvi Öztorun, H., Çınar, E., Turgut, T., Mut Sürmeli, D., Bahşi, R., Atmış, V., . . . Aras, S. (2018). The impact of treatment for iron deficiency and iron deficiency anemia on nutritional status, physical performance, and cognitive function in geriatric patients. *European Geriatric Medicine, 9*(4), 493-500. doi:10.1007/s41999-018-0065-z

Seril, D. N., Liao, J., Ho, K.-L. K., Warsi, A., Yang, C. S., & Yang, G.-Y. (2002). Dietary iron supplementation enhances DSS-induced colitis and associated colorectal carcinoma development in mice. *Digestive diseases and sciences, 47*(6), 1266-1278.

Serre-Miranda, C., Roque, S., Santos, N. C., Portugal-Nunes, C., Costa, P., Palha, J. A., . . . Correia-Neves, M. (2015). Effector memory CD4(+) T cells are associated with cognitive performance in a senior population. *Neurol Neuroimmunol Neuroinflamm, 2*(1), e54. doi:10.1212/nxi.0000000000000054

Shah, R. C., Wilson, R. S., Tang, Y., Dong, X., Murray, A., & Bennett, D. A. (2009). Relation of hemoglobin to level of cognitive function in older persons. *Neuroepidemiology, 32*(1), 40-46. doi:10.1159/000170905

Sharma, R., Stanek, J. R., Koch, T. L., Grooms, L., & O'Brien, S. H. (2016). Intravenous iron therapy in non-anemic iron-deficient menstruating adolescent females with fatigue. *Am J Hematol, 91*(10), 973-977. doi:10.1002/ajh.24461

Shayeghi, M., Latunde-Dada, G. O., Oakhill, J. S., Laftah, A. H., Takeuchi, K., Halliday, N., . . . McKie, A. T. (2005). Identification of an intestinal heme transporter. *Cell, 122*(5), 789-801. doi:10.1016/j.cell.2005.06.025

Sheikh, M., Hantoushzadeh, S., Shariat, M., Farahani, Z., & Ebrahiminasab, O. (2017). The efficacy of early iron supplementation on postpartum depression, a randomized double-blind placebo-controlled trial. *Eur J Nutr, 56*(2), 901-908. doi:10.1007/s00394-015-1140-6

Shukla, A., Agarwal, K. N., Chansuria, J. P., & Taneja, V. (1989). Effect of latent iron deficiency on 5-hydroxytryptamine metabolism in rat brain. *J Neurochem, 52*(3), 730-735. doi:10.1111/j.1471-4159.1989.tb02515.x

Siddappa, A. M., Georgieff, M. K., Wewerka, S., Worwa, C., Nelson, C. A., & Deregnier, R. A. (2004). Iron deficiency alters auditory recognition memory in newborn infants of diabetic mothers. *Pediatr Res, 55*(6), 1034-1041. doi:10.1203/01.pdr.0000127021.38207.62

Silva, B., & Faustino, P. (2015). An overview of molecular basis of iron metabolism regulation and the associated pathologies. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1852*(7), 1347-1359. doi:https://doi.org/10.1016/j.bbadis.2015.03.011

Silvestri, L., Nai, A., Dulja, A., & Pagani, A. (2019). Hepcidin and the BMP-SMAD pathway: An unexpected liaison. *Vitam Horm, 110*, 71-99. doi:10.1016/bs.vh.2019.01.004

Simakajornboon, N., Gozal, D., Vlasic, V., Mack, C., Sharon, D., & McGinley, B. M. (2003). Periodic limb movements in sleep and iron status in children. *Sleep, 26*(6), 735-738. doi:10.1093/sleep/26.6.735

Sinclair, L. M., & Hinton, P. S. (2005). Prevalence of iron deficiency with and without anemia in recreationally active men and women. *J Am Diet Assoc, 105*(6), 975-978. doi:10.1016/j.jada.2005.03.005

Singh, K., Fong, Y. F., & Kuperan, P. (1998). A comparison between intravenous iron polymaltose complex (Ferrum Hausmann) and oral ferrous fumarate in the treatment of iron deficiency anaemia in pregnancy. *Eur J Haematol, 60*(2), 119-124. doi:10.1111/j.1600-0609.1998.tb01008.x

Siqueiros-Cendón, T., Arévalo-Gallegos, S., Iglesias-Figueroa, B. F., García-Montoya, I. A., Salazar-Martínez, J., & Rascón-Cruz, Q. (2014). Immunomodulatory effects of lactoferrin. *Acta pharmacologica Sinica, 35*(5), 557-566. doi:10.1038/aps.2013.200

Skovlund, C. W., Mørch, L. S., Kessing, L. V., & Lidegaard, Ø. (2016). Association of Hormonal Contraception With Depression. *JAMA Psychiatry, 73*(11), 1154-1162. doi:10.1001/jamapsychiatry.2016.2387

Slauch, J. M. (2011). How does the oxidative burst of macrophages kill bacteria? Still an open question. *Molecular microbiology, 80*(3), 580-583. doi:10.1111/j.1365-2958.2011.07612.x

Soleimani, N., & abbaszadeh, N. (2011). Relationship Between Anaemia, Caused from the Iron Deficiency, and Academic Achievement Among Third Grade High School Female Students. *Procedia - Social and Behavioral Sciences, 29*, 1877-1884. doi:https://doi.org/10.1016/j.sbspro.2011.11.437

Solmaz, S., Asma, S., Aygün, B., oğlu, Ç., Korur, A., Kalaycı, H., . . . Ozdogu, H. (2016). An Overlooked Side Effect of Iron Treatment: Changes in Menstruation: Soner S et al. An Overlooked Side Effect of Iron Treatment. *International Journal of Hematology Research, 2*, 120-123. doi:10.17554/j.issn.2409-3548.2015.01.26

Solomons, N. W., & Jacob, R. A. (1981). Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. *The American journal of clinical nutrition, 34*(4), 475-482. doi:10.1093/ajcn/34.4.475

Soppi, E. T. (2018). Iron deficiency without anemia – a clinical challenge. *Clinical Case Reports, 6*(6), 1082-1086. doi:10.1002/ccr3.1529

Sørensen, E., Grau, K., Berg, T., Simonsen, A. C., Magnussen, K., Erikstrup, C., . . . Ullum, H. (2012). A genetic risk factor for low serum ferritin levels in Danish blood donors. *Transfusion, 52*(12), 2585-2589. doi:10.1111/j.1537-2995.2012.03629.x

Sperling, R. (2007). Functional MRI studies of associative encoding in normal aging, mild cognitive impairment, and Alzheimer's disease. *Annals of the New York Academy of Sciences, 1097*(1), 146-155.

Sperling, R., Greve, D., Dale, A., Killiany, R., Holmes, J., Rosas, H. D., . . . Lake, S. (2002). Functional MRI detection of pharmacologically induced memory impairment. *Proceedings of the National Academy of Sciences, 99*(1), 455-460.

Srinivasan, S., & Avadhani, N. G. (2012). Cytochrome c oxidase dysfunction in oxidative stress. *Free radical biology & medicine, 53*(6), 1252-1263. doi:10.1016/j.freeradbiomed.2012.07.021

Staley, J. K., Sanacora, G., Tamagnan, G., Maciejewski, P. K., Malison, R. T., Berman, R. M., . . . Innis, R. B. (2006). Sex differences in diencephalon serotonin transporter availability in major depression. *Biological Psychiatry, 59*(1), 40-47. doi:10.1016/j.biopsych.2005.06.012

Stekel, A., Olivares, M., Pizarro, F., Chadud, P., Lopez, I., & Amar, M. (1986). Absorption of fortification iron from milk formulas in infants. *The American journal of clinical nutrition, 43*(6), 917-922. doi:10.1093/ajcn/43.6.917

Sterr, A., Ebajemito, J. K., Mikkelsen, K. B., Bonmati-Carrion, M. A., Santhi, N., Della Monica, C., . . . DeVos, M. (2018). Sleep EEG Derived From Behind-the-Ear Electrodes (cEEGrid) Compared to Standard Polysomnography: A Proof of Concept Study. *Frontiers in human neuroscience, 12*, 452-452. doi:10.3389/fnhum.2018.00452

Stoecker, B. J., Abebe, Y., Hubbs-Tait, L., Kennedy, T. S., Gibson, R. S., Arbide, I., . . . Hambidge, K. M. (2009). Zinc status and cognitive function of pregnant women in Southern Ethiopia. *European Journal of Clinical Nutrition, 63*(7), 916-918. doi:10.1038/ejcn.2008.77

Stoffel, N. U., Cercamondi, C. I., Brittenham, G., Zeder, C., Geurts-Moespot, A. J., Swinkels, D. W., . . . Zimmermann, M. B. (2017). Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomised controlled trials. *Lancet Haematol, 4*(11), e524-e533. doi:10.1016/s2352-3026(17)30182-5

Stoffel, N. U., Zeder, C., Brittenham, G. M., Moretti, D., & Zimmermann, M. B. (2019). Iron absorption from supplements is greater with alternate day than with consecutive day dosing in iron-deficient anemic women. *Haematologica*. doi:10.3324/haematol.2019.220830

Stoltzfus, R. J., Kvalsvig, J. D., Chwaya, H. M., Montresor, A., Albonico, M., Tielsch, J. M., . . . Pollitt, E. (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. *BMJ (Clinical research ed.), 323*(7326), 1389. doi:10.1136/bmj.323.7326.1389

Stone, A. A., Shiffman, S., Schwartz, J. E., Broderick, J. E., & Hufford, M. R. (2003). Patient compliance with paper and electronic diaries. *Control Clin Trials, 24*(2), 182-199. doi:10.1016/s0197-2456(02)00320-3

Stonehouse, W., Conlon, C. A., Podd, J., Hill, S. R., Minihane, A. M., Haskell, C., & Kennedy, D. (2013). DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. *The American journal of clinical nutrition, 97*(5), 1134-1143. doi:10.3945/ajcn.112.053371

Strangman, G., Boas, D. A., & Sutton, J. P. (2002). Non-invasive neuroimaging using near-infrared light. *Biological Psychiatry, 52*(7), 679-693. doi:https://doi.org/10.1016/S0006-3223(02)01550-0

Strangman, G., Culver, J. P., Thompson, J. H., & Boas, D. A. (2002). A Quantitative Comparison of Simultaneous BOLD fMRI and NIRS Recordings during Functional Brain Activation. *NeuroImage, 17*(2), 719-731. doi:https://doi.org/10.1006/nimg.2002.1227

Strasser, B., & Fuchs, D. (2015). Role of physical activity and diet on mood, behavior, and cognition. *Neurology, Psychiatry and Brain Research, 21*(3), 118-126. doi:https://doi.org/10.1016/j.npbr.2015.07.002

Streck, E. L., Goncalves, C. L., Furlanetto, C. B., Scaini, G., Dal-Pizzol, F., & Quevedo, J. (2014). Mitochondria and the central nervous system: searching for a pathophysiological basis of psychiatric disorders. *Braz J Psychiatry, 36*(2), 156-167. doi:10.1590/1516-4446-2013-1224

Su, Q., Gu, Y., Yu, B., Yu, F., He, H., Zhang, Q., . . . Liu, L. (2016). Association between serum ferritin concentrations and depressive symptoms among Chinese adults: a population study from the Tianjin Chronic Low-Grade Systemic Inflammation and Health (TCLSIHealth) cohort study. *PloS one, 11*(9), e0162682.

Suchdev, P. S., Williams, A. M., Mei, Z., Flores-Ayala, R., Pasricha, S.-R., Rogers, L. M., & Namaste, S. M. (2017). Assessment of iron status in settings of inflammation:

challenges and potential approaches. *The American journal of clinical nutrition, 106*(Suppl 6), 1626S-1633S. doi:10.3945/ajcn.117.155937

Sundstrom Poromaa, I., & Gingnell, M. (2014). Menstrual cycle influence on cognitive function and emotion processing-from a reproductive perspective. *Front Neurosci, 8*, 380. doi:10.3389/fnins.2014.00380

Sungthong, R., Mo-suwan, L., & Chongsuvivatwong, V. (2002). Effects of haemoglobin and serum ferritin on cognitive function in school children. *Asia Pac J Clin Nutr, 11*(2), 117-122. doi:10.1046/j.1440-6047.2002.00272.x

Sur, S., & Sinha, V. K. (2009). Event-related potential: An overview. *Industrial psychiatry journal, 18*(1), 70-73. doi:10.4103/0972-6748.57865

Švob Štrac, D., Pivac, N., & Mück-Šeler, D. (2016). The serotonergic system and cognitive function. *Translational neuroscience, 7*(1), 35-49. doi:10.1515/tnsci-2016-0007

Swain, J. H., Penland, J. G., Johnson, L. K., & Hunt, J. R. (2006). Energy, mood and attention did not consistently improve with iron status in non-anemic women with moderate to low iron stores. *The FASEB Journal, 20*(4), A191-A192. doi:10.1096/fasebj.20.4.A191-d

Sweat, V., Starr, V., Bruehl, H., Arentoft, A., Tirsi, A., Javier, E., & Convit, A. (2008). C-reactive protein is linked to lower cognitive performance in overweight and obese women. *Inflammation, 31*(3), 198-207.

Szajewska, H., Ruszczynski, M., & Chmielewska, A. (2010). Effects of iron supplementation in nonanemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials. *The American journal of clinical nutrition, 91*(6), 1684-1690. doi:10.3945/ajcn.2010.29191

Szarfarc, S. C., de Cassana, L. M., Fujimori, E., Guerra-Shinohara, E. M., & de Oliveira, I. M. (2001). Relative effectiveness of iron bis-glycinate chelate (Ferrochel) and ferrous sulfate in the control of iron deficiency in pregnant women. *Arch Latinoam Nutr, 51*(1 Suppl 1), 42-47.

Takahashi, A., Flanigan, M. E., McEwen, B. S., & Russo, S. J. (2018). Aggression, Social Stress, and the Immune System in Humans and Animal Models. *Frontiers in Behavioral Neuroscience, 12*(56). doi:10.3389/fnbeh.2018.00056

Takeuchi, H., Taki, Y., Nouchi, R., Yokoyama, R., Kotozaki, Y., Nakagawa, S., . . . Kawashima, R. (2018). Shorter sleep duration and better sleep quality are associated with greater tissue density in the brain. *Scientific reports, 8*(1), 5833. doi:10.1038/s41598-018-24226-0

Tamura, T., Goldenberg, R. L., Hou, J., Johnston, K. E., Cliver, S. P., Ramey, S. L., & Nelson, K. G. (2002). Cord serum ferritin concentrations and mental and

psychomotor development of children at five years of age. *The Journal of Pediatrics, 140*(2), 165-170.

Tang, Y. M., Chen, X. Z., Li, G. R., Zhou, R. H., Ning, H., & Yan, H. (2006). [Effects of iron deficiency anemia on immunity and infectious disease in pregnant women]. *Wei Sheng Yan Jiu, 35*(1), 79-81.

Telarović, S., & Čondić, L. (2019). Frequency of iron deficiency anemia in pregnant and non-pregnant women suffering from restless legs syndrome. *Hematology, 24*(1), 263-267. doi:10.1080/16078454.2018.1560935

Tempel, M., Chawla, A., Messina, C., & Celiker, M. Y. (2013). Effects of omeprazole on iron absorption: preliminary study. *Turk J Haematol, 30*(3), 307-310. doi:10.4274/tjh.2013.0042

Texel, S. J., Camandola, S., Ladenheim, B., Rothman, S. M., Mughal, M. R., Unger, E. L., . . . Mattson, M. P. (2012). Ceruloplasmin deficiency results in an anxiety phenotype involving deficits in hippocampal iron, serotonin, and BDNF. *J Neurochem, 120*(1), 125-134. doi:10.1111/j.1471-4159.2011.07554.x

Thomas, C., & Lumb, A. B. (2012). Physiology of haemoglobin. *Continuing Education in Anaesthesia Critical Care & Pain, 12*(5), 251-256. doi:10.1093/bjaceaccp/mks025

Thompson, J., Biggs, B. A., & Pasricha, S. R. (2013). Effects of daily iron supplementation in 2- to 5-year-old children: systematic review and meta-analysis. *Pediatrics, 131*(4), 739-753. doi:10.1542/peds.2012-2256

Tobin, B. W., & Beard, J. L. (1989). Interactions of iron deficiency and exercise training in male Sprague-Dawley rats: ferrokinetics and hematology. *J Nutr, 119*(9), 1340-1347. doi:10.1093/jn/119.9.1340

Toblli, J. E., & Brignoli, R. (2007). Iron(III)-hydroxide polymaltose complex in iron deficiency anemia / review and meta-analysis. *Arzneimittelforschung, 57*(6a), 431-438. doi:10.1055/s-0031-1296692

Todorich, B., Pasquini, J. M., Garcia, C. I., Paez, P. M., & Connor, J. R. (2009). Oligodendrocytes and myelination: the role of iron. *Glia, 57*(5), 467-478. doi:10.1002/glia.20784

Toffol, E., Heikinheimo, O., Koponen, P., Luoto, R., & Partonen, T. (2012). Further evidence for lack of negative associations between hormonal contraception and mental health. *Contraception, 86*(5), 470-480.

Tolkien, Z., Stecher, L., Mander, A. P., Pereira, D. I. A., & Powell, J. J. (2015). Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. *PLoS One, 10*(2), e0117383-e0117383. doi:10.1371/journal.pone.0117383

Torres, M. A., Lobo, N. F., Sato, K., & Queiroz Sde, S. (1996). [Fortification of fluid milk for the prevention and treatment of iron deficiency anemia in children under 4 years of age]. *Rev Saude Publica, 30*(4), 350-357. doi:10.1590/s0034-89101996000400008

Toxqui, L., Pérez-Granados, A. M., Blanco-Rojo, R., Wright, I., & Vaquero, M. P. (2014). A simple and feasible questionnaire to estimate menstrual blood loss: relationship with hematological and gynecological parameters in young women. *BMC Women's Health, 14*(1), 71. doi:10.1186/1472-6874-14-71

Travica, N., Ried, K., Sali, A., Hudson, I., Scholey, A., & Pipingas, A. (2019). Plasma Vitamin C Concentrations and Cognitive Function: A Cross-Sectional Study. *Frontiers in aging neuroscience, 11*, 72-72. doi:10.3389/fnagi.2019.00072

Travica, N., Ried, K., Sali, A., Scholey, A., Hudson, I., & Pipingas, A. (2017). Vitamin C Status and Cognitive Function: A Systematic Review. *Nutrients, 9*(9), 960. doi:10.3390/nu9090960

Troost, F.J., Brummer, R-J.M., Dainty, J.R., Hoogewerff, J.A., Bull, V.J., & Saris, W.H.M. (2003). Iron supplements inhibit zinc but not copper absorption in vivo in ileostomy subjects. *Am J Clin Nutr, 78*(5), 1018-1023

Trotti, L. M., & Becker, L. A. (2019). Iron for the treatment of restless legs syndrome. *The Cochrane database of systematic reviews, 1*(1), CD007834-CD007834. doi:10.1002/14651858.CD007834.pub3

Tussing-Humphreys, L., Pusatcioglu, C., Nemeth, E., & Braunschweig, C. (2012). Rethinking iron regulation and assessment in iron deficiency, anemia of chronic disease, and obesity: introducing hepcidin. *J Acad Nutr Diet, 112*(3), 391-400. doi:10.1016/j.jada.2011.08.038

Ullah, M. F., Ahmad, A., Bhat, S. H., Abu-Duhier, F. M., Barreto, G. E., & Ashraf, G. M. (2019). Impact of sex differences and gender specificity on behavioral characteristics and pathophysiology of neurodegenerative disorders. *Neuroscience & Biobehavioral Reviews, 102*, 95-105. doi:https://doi.org/10.1016/j.neubiorev.2019.04.003

Uludag, K., Dubowitz, D. J., Yoder, E. J., Restom, K., Liu, T. T., & Buxton, R. B. (2004). Coupling of cerebral blood flow and oxygen consumption during physiological activation and deactivation measured with fMRI. *NeuroImage, 23*(1), 148-155. doi:10.1016/j.neuroimage.2004.05.013

Umbreit, J. (2005). Iron deficiency: a concise review. *Am J Hematol, 78*(3), 225-231. doi:10.1002/ajh.20249

Unger, E. L., Hurst, A. R., Georgieff, M. K., Schallert, T., Rao, R., Connor, J. R., . . . Felt, B. (2012). Behavior and monoamine deficits in prenatal and perinatal iron deficiency are not corrected by early postnatal moderate-iron or high-iron diets in rats. *J Nutr, 142*(11), 2040-2049. doi:10.3945/jn.112.162198

Vahdat Shariatpanaahi, M., Vahdat Shariatpanaahi, Z., Moshtaaghi, M., Shahbaazi, S. H., & Abadi, A. (2007). The relationship between depression and serum ferritin level. *European Journal of Clinical Nutrition, 61*(4), 532-535. doi:10.1038/sj.ejcn.1602542

Vaucher, P., Druais, P.-L., Waldvogel, S., & Favrat, B. (2012). Effect of iron supplementation on fatigue in nonanemic menstruating women with low ferritin: a randomized controlled trial. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 184*(11), 1247-1254.

Vazquez, A. L., Masamoto, K., Fukuda, M., & Kim, S.-G. (2010). Cerebral oxygen delivery and consumption during evoked neural activity. *Frontiers in neuroenergetics, 2*, 11-11. doi:10.3389/fnene.2010.00011

Verdon, F., Burnand, B., Stubi, C. L. F., Bonard, C., Graff, M., Michaud, A., . . . Favrat, B. (2003). Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *BMJ (Clinical research ed.), 326*(7399), 1124-1124. doi:10.1136/bmj.326.7399.1124

Verner, A. M., Manderson, J., Lappin, T. R. J., McCance, D. R., Halliday, H. L., & Sweet, D. G. (2007). Influence of maternal diabetes mellitus on fetal iron status. *Archives of disease in childhood. Fetal and neonatal edition, 92*(5), F399-F401. doi:10.1136/adc.2006.097279

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Kales, A., Tyson, K., & Chrousos, G. P. (1997). Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab, 82*(5), 1313-1316. doi:10.1210/jcem.82.5.3950

Villeda, S. A., Luo, J., Mosher, K. I., Zou, B., Britschgi, M., Bieri, G., . . . Wyss-Coray, T. (2011). The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature, 477*(7362), 90-94. doi:10.1038/nature10357

Villeda, S. A., Plambeck, K. E., Middeldorp, J., Castellano, J. M., Mosher, K. I., Luo, J., . . . Wyss-Coray, T. (2014). Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat Med, 20*(6), 659-663. doi:10.1038/nm.3569

Walker, E. M., Jr., & Walker, S. M. (2000). Effects of iron overload on the immune system. *Ann Clin Lab Sci, 30*(4), 354-365.

Wang, B., Zhan, S., Gong, T., & Lee, L. (2013). Iron therapy for improving psychomotor development and cognitive function in children under the age of three with iron deficiency anaemia. *Cochrane Database of Systematic Reviews*(6). doi:10.1002/14651858.CD001444.pub2

Wang, J., & Pantopoulos, K. (2011). Regulation of cellular iron metabolism. *The Biochemical journal, 434*(3), 365-381. doi:10.1042/BJ20101825

Wang, J., Song, N., Jiang, H., Wang, J., & Xie, J. (2013). Pro-inflammatory cytokines modulate iron regulatory protein 1 expression and iron transportation through reactive oxygen/nitrogen species production in ventral mesencephalic neurons. *Biochimica et biophysica acta, 1832*(5), 618-625. doi:10.1016/j.bbadis.2013.01.021

Wang, Q., Li, S., Tang, X., Liang, L., Wang, F., & Du, H. (2019). Lipocalin 2 Protects Against Escherichia coli Infection by Modulating Neutrophil and Macrophage Function. *Frontiers in immunology, 10*, 2594-2594. doi:10.3389/fimmu.2019.02594

Wang, W., Bourgeois, T., Klima, J., Berlan, E. D., Fischer, A. N., & O'Brien, S. H. (2013). Iron deficiency and fatigue in adolescent females with heavy menstrual bleeding. *Haemophilia, 19*(2), 225-230. doi:10.1111/hae.12046

Wang, Y., Huang, L., Zhang, L., Qu, Y., & Mu, D. (2017). Iron Status in Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-Analysis. *PLoS One, 12*(1), e0169145-e0169145. doi:10.1371/journal.pone.0169145

Ward, K. L., Tkac, I., Jing, Y., Felt, B., Beard, J., Connor, J., . . . Rao, R. (2007). Gestational and lactational iron deficiency alters the developing striatal metabolome and associated behaviors in young rats. *The Journal of nutrition, 137*(4), 1043-1049. doi:10.1093/jn/137.4.1043

Ward, R. J., Crichton, R. R., Taylor, D. L., Della Corte, L., Srai, S. K., & Dexter, D. T. (2011). Iron and the immune system. *J Neural Transm (Vienna), 118*(3), 315-328. doi:10.1007/s00702-010-0479-3

Ward, R. J., Zucca, F. A., Duyn, J. H., Crichton, R. R., & Zecca, L. (2014). The role of iron in brain ageing and neurodegenerative disorders. *The Lancet Neurology, 13*(10), 1045-1060. doi:https://doi.org/10.1016/S1474-4422(14)70117-6

Ware, J., Jr., Kosinski, M., & Keller, S. D. (1996). A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care, 34*(3), 220-233. doi:10.1097/00005650-199603000-00003

Warshafsky, C., Pudwell, J., Walker, M., Wen, S.-W., & Smith, G. N. (2016). Prospective assessment of neurodevelopment in children following a pregnancy complicated by severe pre-eclampsia. *BMJ Open, 6*(7), e010884. doi:10.1136/bmjopen-2015-010884

Wassef, A., Nguyen, Q. D., & St-Andre, M. (2019). Anaemia and depletion of iron stores as risk factors for postpartum depression: a literature review. *J Psychosom Obstet Gynaecol, 40*(1), 19-28. doi:10.1080/0167482x.2018.1427725

Wazir, S.M., & Ghobrial, I. (2017). Copper deficiency, a new triad: anemia, leucopenia, and myeloneuropathy. *J Community Hosp Intern Med Perspect, 7*(4), 265-268

Weber, D., & Skirbekk, V. (2014). *The Educational Effect on Cognitive Functioning: National versus Individual Educational Attainment*.

Wenger, M. J., DellaValle, D. M., Murray-Kolb, L. E., & Haas, J. D. (2019). Effect of iron deficiency on simultaneous measures of behavior, brain activity, and energy expenditure in the performance of a cognitive task. *Nutritional neuroscience, 22*(3), 196-206.

Wenger, M. J., Murray-Kolb, L. E., Nevins, J. E., Venkatramanan, S., Reinhart, G. A., Wesley, A., & Haas, J. D. (2017). Consumption of a Double-Fortified Salt Affects Perceptual, Attentional, and Mnemonic Functioning in Women in a Randomized Controlled Trial in India. *J Nutr, 147*(12), 2297-2308. doi:10.3945/jn.117.251587

Wenger, M. J., Rhoten, S. E., Murray-Kolb, L. E., Scott, S. P., Boy, E., Gahutu, J.-B., & Haas, J. D. (2019). Changes in Iron Status Are Related to Changes in Brain Activity and Behavior in Rwandan Female University Students: Results from a Randomized Controlled Efficacy Trial Involving Iron-Biofortified Beans. *The Journal of nutrition, 149*(4), 687-697. doi:10.1093/jn/nxy265

Wenninger, J., Meinitzer, A., Holasek, S., Schnedl, W. J., Zelzer, S., Mangge, H., . . . Enko, D. (2019). Associations between tryptophan and iron metabolism observed in individuals with and without iron deficiency. *Scientific reports, 9*(1), 14548. doi:10.1038/s41598-019-51215-8

Whitfield, J. B., Treloar, S., Zhu, G., Powell, L. W., & Martin, N. G. (2003). Relative importance of female-specific and non-female-specific effects on variation in iron stores between women. *British journal of haematology, 120*(5), 860-866. doi:10.1046/j.1365-2141.2003.04224.x

Wieringa, F. T., Dijkhuizen, M. A., Fiorentino, M., Laillou, A., & Berger, J. (2015). Determination of zinc status in humans: which indicator should we use? *Nutrients, 7*(5), 3252-3263. doi:10.3390/nu7053252

Wightman, E. L., Haskell-Ramsay, C. F., Reay, J. L., Williamson, G., Dew, T., Zhang, W., & Kennedy, D. O. (2015). The effects of chronic trans-resveratrol supplementation on aspects of cognitive function, mood, sleep, health and cerebral blood flow in healthy, young humans. *Br J Nutr, 114*(9), 1427-1437. doi:10.1017/s0007114515003037

Wightman, E. L., Jackson, P. A., Khan, J., Forster, J., Heiner, F., Feistel, B., . . . Kennedy, D. O. (2018). The Acute and Chronic Cognitive and Cerebral Blood Flow Effects of a Sideritis scardica (Greek Mountain Tea) Extract: A Double Blind, Randomized, Placebo Controlled, Parallel Groups Study in Healthy Humans. *Nutrients, 10*(8), 955. doi:10.3390/nu10080955

Willemetz, A., Beatty, S., Richer, E., Rubio, A., Auriac, A., Milkereit, R. J., . . . Canonne-Hergaux, F. (2017). Iron- and Hepcidin-Independent Downregulation of the Iron Exporter Ferroportin in Macrophages during Salmonella Infection. *Frontiers in immunology, 8*, 498-498. doi:10.3389/fimmu.2017.00498

Wolf, S. A., Steiner, B., Akpinarli, A., Kammertoens, T., Nassenstein, C., Braun, A., . . . Kempermann, G. (2009). CD4-positive T lymphocytes provide a neuroimmunological link in the control of adult hippocampal neurogenesis. *J Immunol, 182*(7), 3979-3984. doi:10.4049/jimmunol.0801218

Woods, A., Garvican-Lewis, L. A., Saunders, P. U., Lovell, G., Hughes, D., Fazakerley, R., . . . Thompson, K. G. (2014). Four weeks of IV iron supplementation reduces perceived fatigue and mood disturbance in distance runners. *PLoS One, 9*(9), e108042. doi:10.1371/journal.pone.0108042

Wu, Y., Chen, M., & Jiang, J. (2019). Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signaling. *Mitochondrion, 49*, 35-45. doi:10.1016/j.mito.2019.07.003

Wullschleger, S., Loewith, R., & Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell, 124*(3), 471-484.

Xie, R., Xie, H., Krewski, D., & He, G. (2018). Plasma concentrations of neurotransmitters and postpartum depression. *Zhong Nan Da Xue Xue Bao Yi Xue Ban, 43*(3), 274-281. doi:10.11817/j.issn.1672-7347.2018.03.007

Xu, J., Jia, Z., Knutson, M. D., & Leeuwenburgh, C. (2012). Impaired iron status in aging research. *International journal of molecular sciences, 13*(2), 2368-2386.

Yang, M., Collis, C., Kelly, M., Diplock, A., & Rice-Evans, C. (1999). Do iron and vitamin C co-supplementation influence platelet function or LDL oxidizability in healthy volunteers? *European Journal of Clinical Nutrition, 53*(5), 367-374.

Yang, R., Gao, C., Wu, X., Yang, J., Li, S., & Cheng, H. (2016). Decreased functional connectivity to posterior cingulate cortex in major depressive disorder. *Psychiatry Research: Neuroimaging, 255*, 15-23. doi:https://doi.org/10.1016/j.pscychresns.2016.07.010

Yasa, B., Agaoglu, L., & Unuvar, E. (2011). Efficacy, Tolerability, and Acceptability of Iron Hydroxide Polymaltose Complex versus Ferrous Sulfate: A Randomized Trial in Pediatric Patients with Iron Deficiency Anemia. *Int J Pediatr, 2011*, 524520. doi:10.1155/2011/524520

Yavuz, B. B., Cankurtaran, M., Haznedaroglu, I. C., Halil, M., Ulger, Z., Altun, B., & Ariogul, S. (2012). Iron deficiency can cause cognitive impairment in geriatric patients. *The journal of nutrition, health & aging, 16*(3), 220-224. doi:10.1007/s12603-011-0351-7

Yazici, K. U., Yazici, I. P., & Ustundag, B. (2019). Increased Serum Hepcidin Levels in Children and Adolescents with Attention Deficit Hyperactivity Disorder. *Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology, 17*(1), 105-112. doi:10.9758/cpn.2019.17.1.105

Yetkin-Arik, B., Vogels, I. M. C., Nowak-Sliwinska, P., Weiss, A., Houtkooper, R. H., Van Noorden, C. J. F., . . . Schlingemann, R. O. (2019). The role of glycolysis and mitochondrial respiration in the formation and functioning of endothelial tip cells during angiogenesis. *Scientific reports, 9*(1), 12608. doi:10.1038/s41598-019-48676-2

Yi, S., Nanri, A., Poudel-Tandukar, K., Nonaka, D., Matsushita, Y., Hori, A., & Mizoue, T. (2011). Association between serum ferritin concentrations and depressive symptoms in Japanese municipal employees. *Psychiatry Res, 189*(3), 368-372. doi:10.1016/j.psychres.2011.03.009

Yildiz, G., Senturk, M. B., Yildiz, P., Cakmak, Y., Budak, M. S., & Cakar, E. (2017). Serum serotonin, leptin, and adiponectin changes in women with postpartum depression: controlled study. *Archives of Gynecology and Obstetrics, 295*(4), 853-858. doi:10.1007/s00404-017-4313-0

Yokoi, K., & Konomi, A. (2017). Iron deficiency without anaemia is a potential cause of fatigue: meta-analyses of randomised controlled trials and cross-sectional studies. *Br J Nutr, 117*(10), 1422-1431. doi:10.1017/s0007114517001349

Youdim, M. B., Ben-Shachar, D., & Yehuda, S. (1989). Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. *The American journal of clinical nutrition, 50*(3), 607-617.

Youdim, M. B., & Green, A. R. (1978). Iron deficiency and neurotransmitter synthesis and function. *Proc Nutr Soc, 37*(2), 173-179. doi:10.1079/pns19780022

Youdim, M. B., Sills, M. A., Heydorn, W. E., Creed, G. J., & Jacobowitz, D. M. (1986). Iron deficiency alters discrete proteins in rat caudate nucleus and nucleus accumbens. *J Neurochem, 47*(3), 794-799. doi:10.1111/j.1471-4159.1986.tb00681.x

Young, I., Parker, H. M., Rangan, A., Prvan, T., Cook, R. L., Donges, C. E., . . . O'Connor, H. T. (2018). Association between Haem and Non-Haem Iron Intake and Serum Ferritin in Healthy Young Women. *Nutrients, 10*(1). doi:10.3390/nu10010081

Yu, G. S., Steinkirchner, T. M., Rao, G. A., & Larkin, E. C. (1986). Effect of prenatal iron deficiency on myelination in rat pups. *Am J Pathol, 125*(3), 620-624.

Yu, S., Feng, Y., Shen, Z., & Li, M. (2011). Diet supplementation with iron augments brain oxidative stress status in a rat model of psychological stress. *Nutrition, 27*(10), 1048-1052.

Zanto, T. P., & Gazzaley, A. (2009). Neural suppression of irrelevant information underlies optimal working memory performance. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 29*(10), 3059-3066. doi:10.1523/jneurosci.4621-08.2009

Zariwala, M. G., Somavarapu, S., Farnaud, S., & Renshaw, D. (2013). Comparison study of oral iron preparations using a human intestinal model. *Scientia pharmaceutica, 81*(4), 1123-1139. doi:10.3797/scipharm.1304-03

Zhang, S., Osumi, H., Uchizawa, A., Hamada, H., Park, I., Suzuki, Y., . . . Tokuyama, K. (2020). Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiological reports, 8*(2), e14353-e14353. doi:10.14814/phy2.14353

Zheng, W., Nichol, H., Liu, S., Cheng, Y.-C. N., & Haacke, E. M. (2013). Measuring iron in the brain using quantitative susceptibility mapping and X-ray fluorescence imaging. *NeuroImage, 78*, 68-74. doi:10.1016/j.neuroimage.2013.04.022

Zhu, A., Kaneshiro, M., & Kaunitz, J. D. (2010). Evaluation and treatment of iron deficiency anemia: a gastroenterological perspective. *Digestive diseases and sciences, 55*(3), 548-559. doi:10.1007/s10620-009-1108-6

Zhu, Y. I., & Haas, J. D. (1998). Altered metabolic response of iron-depleted nonanemic women during a 15-km time trial. *Journal of Applied Physiology, 84*(5), 1768-1775.

Zielinski, M. R., McKenna, J. T., & McCarley, R. W. (2016). Functions and Mechanisms of Sleep. *AIMS neuroscience, 3*(1), 67-104. doi:10.3934/Neuroscience.2016.1.67

Zimmermann, M. B., Chassard, C., Rohner, F., N'Goran E, K., Nindjin, C., Dostal, A., . . . Hurrell, R. F. (2010). The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. *The American journal of clinical nutrition, 92*(6), 1406-1415. doi:10.3945/ajcn.110.004564

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British journal of pharmacology, 175*(16), 3190-3199. doi:10.1111/bph.14116

APPENDICES

APPENDIX I: Caffeine consumption questionnaire (CCQ) (BPNRC, Northumbria University)

Portion Sizes Guide:

Example of how to complete:

	Coffee		Cola, mixed cola beverages (no orangeade/lemonade)				Solid chocolate bar e.g. Dairy Milk
							
Breakfast	II			I			I

Please indicate what you would have on a typical day:

	Fresh Brewed Coffee		Instant Coffee		Decaffeinated coffee		Black, green, white tea	Decaffeinated Black, green, white tea	Cocoa drink		Cola, mixed cola beverages (but not orangeade and lemonade)				Lucozade Sport (Still Drink) 500 mL		Lucozade Energy (Fizzy Drink) 500 mL	Red Bull Energy Drink (250 mL)	Monster/ Relentless/ Rockstar Energy Drink (500 mL)	Solid Chocolate Bar	1 Square of Solid Chocolate
															Fuel and Focus	Original and other Varieties					
																					
Breakfast																					
Between breakfast and lunch																					
lunch																					
Between lunch and dinner																					
Dinner																					
After dinner																					
TOTAL																					

APPENDIX II: Menstrual cycle and blood loss questionnaire

Menstrual Cycle Questionnaire

Have you used hormonal contraception in the last 3 months?

□ Yes…

□ Combined pill

□ Progesterone-only pill (POP)

□ Contraceptive injection

□ Contraceptive implant

□ Intrauterine device (IUD)

□ Other _____

□ No

Have you had a period in the last 3 months? (Natural periods or withdrawal bleeds whilst on contraception)

□ Yes

□ No

Date of onset of your last period (day/month/year): _____

Usual n.o. days menstruation +/- how many days: _____

Estimated number of heavy blood loss days during menstruation: _____

Indicate the number and type of pads and/or tampons used at heaviest blood loss day of menstruation, both during the day and night:

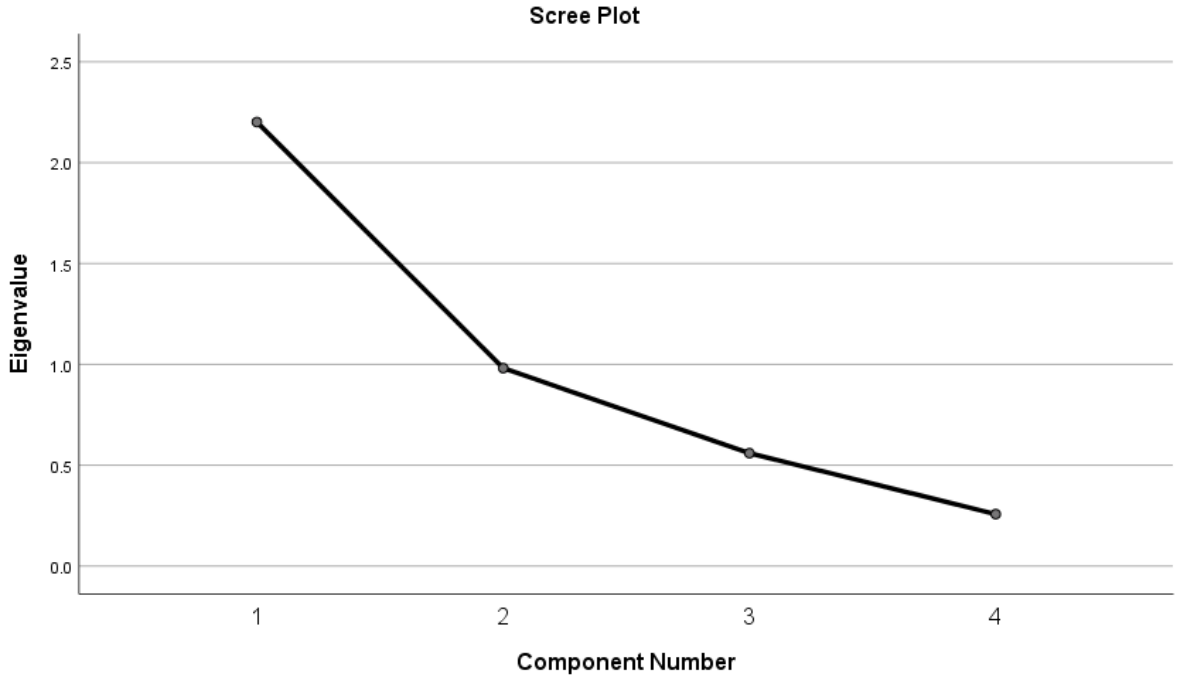
	Number of pads				Number of tampons			
	Mini	Normal	Super	Night/Superplus	Light	Normal	Super	Night/Superplus
Day								
Night								

Estimated date of onset of your next period: _____

APPENDIX III: Principle component analysis for cognitive outcomes

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Episodic Accuracy	230	2.201	55.033	55.033	0.671	264.648	6	<0.001

*only eigenvalues ≥1 are reported



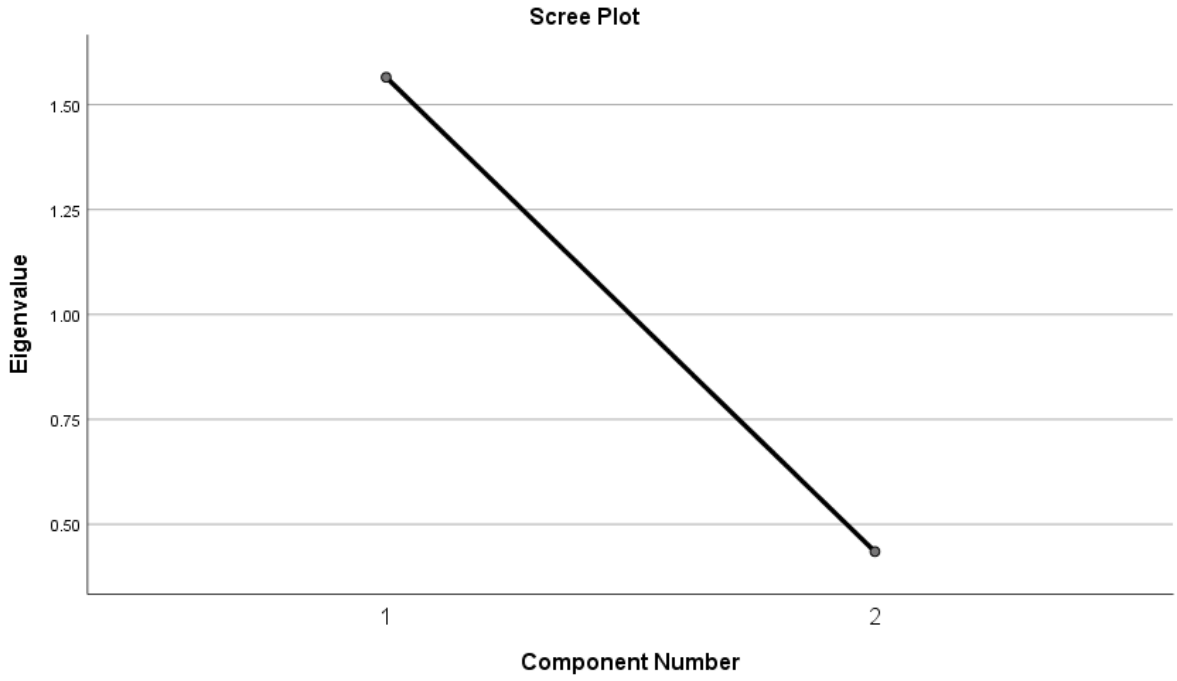
Component Matrix[a]	Component 1
ZIWR_Correct	0.886
ZDWR_Correct	0.884
ZWord_Rec_Correct	0.768
ZPic_Rec_Correct	0.51

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Episodic Speed	230	1.565	78.253	78.253	0.5	87.499	1	<0.001

*only eigenvalues ≥1 are reported



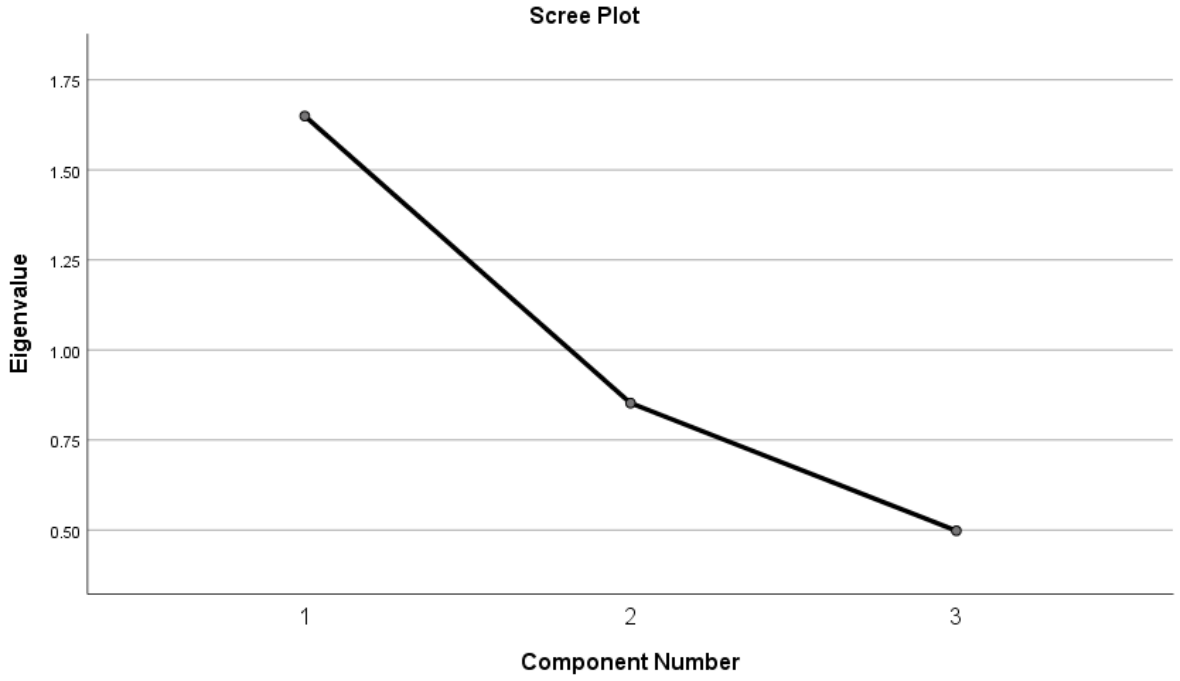
Component Matrix[a]	Component 1
ZPic_Rec_Correct_RT	0.885
ZWord_Rec_Correct_RT	0.885

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Working Memory Accuracy	218	1.649	54.98	54.98	0.571	76.613	3	<0.001

*only eigenvalues ≥1 are reported



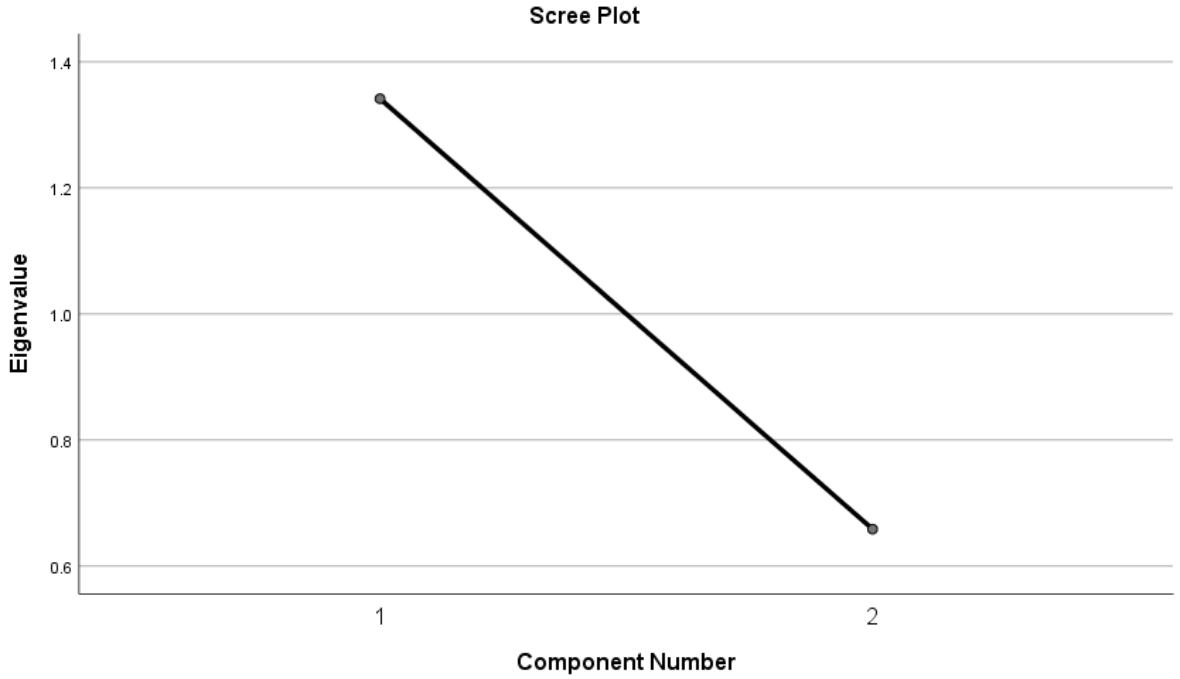
Component Matrix[a]	Component 1
ZZSS7Acc	0.821
ZZSS3Acc	0.819
ZNWM_Correct	0.553

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Attention Accuracy	213	1.341	67.068	67.068	0.500	26.080	1	<0.001

*only eigenvalues ≥1 are reported



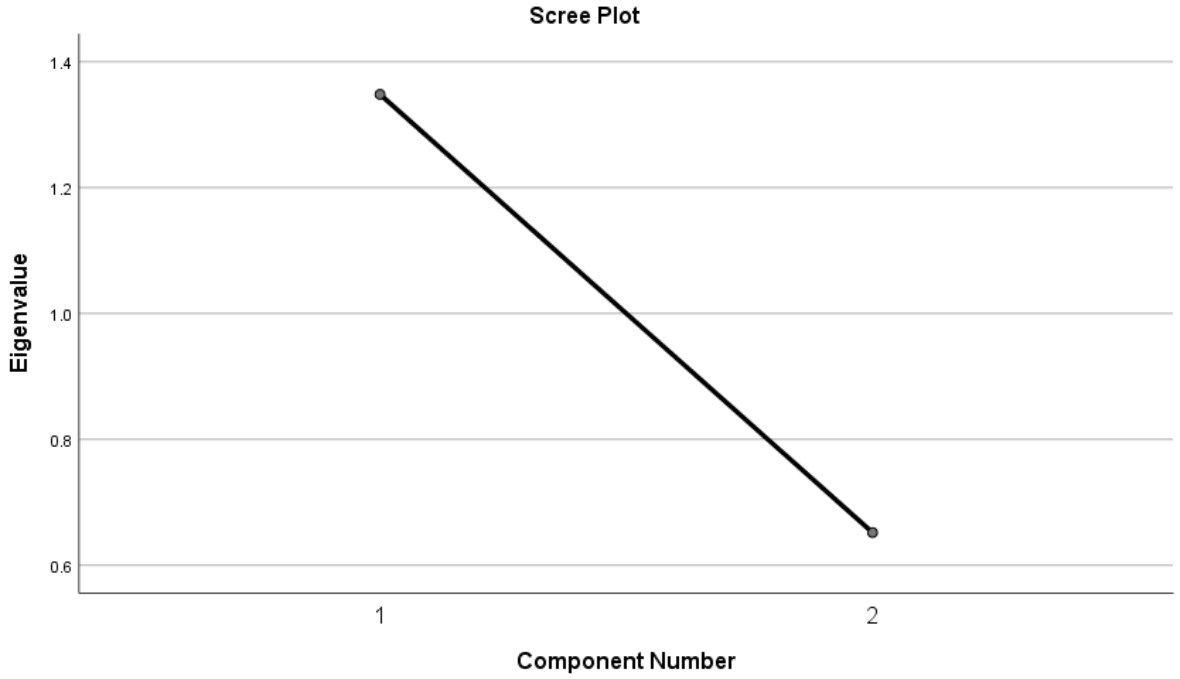
Component Matrix[a]	Component 1
ZZDigVigAcc_err	0.819
ZZRVIPAcc_FA	0.819

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Attention Speed	217	1.348	67.403	67.402	.500	27.182	1	<0.001

*only eigenvalues ≥1 are reported



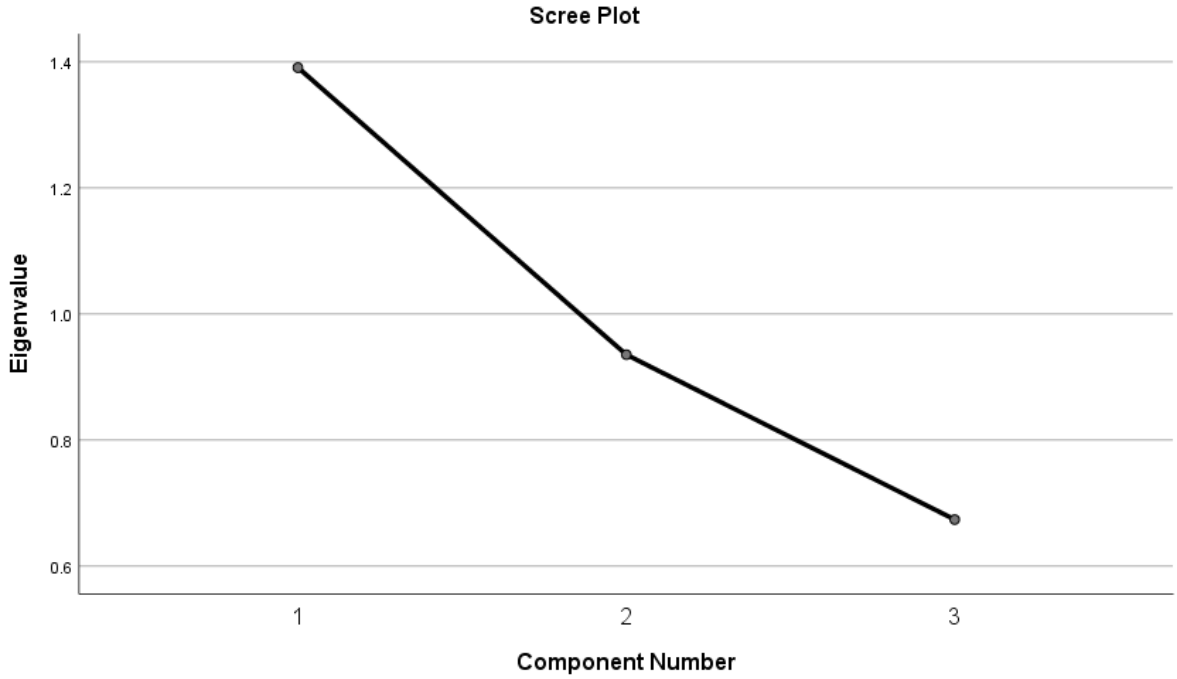
Component Matrix[a]	Component 1
ZDigVig_Correct_RT	0.821
ZRVIP_Correct_RT	0.821

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Executive Function Accuracy	228	1.391	46.358	46.358	0.534	29.521	3	<0.001

*only eigenvalues ≥1 are reported



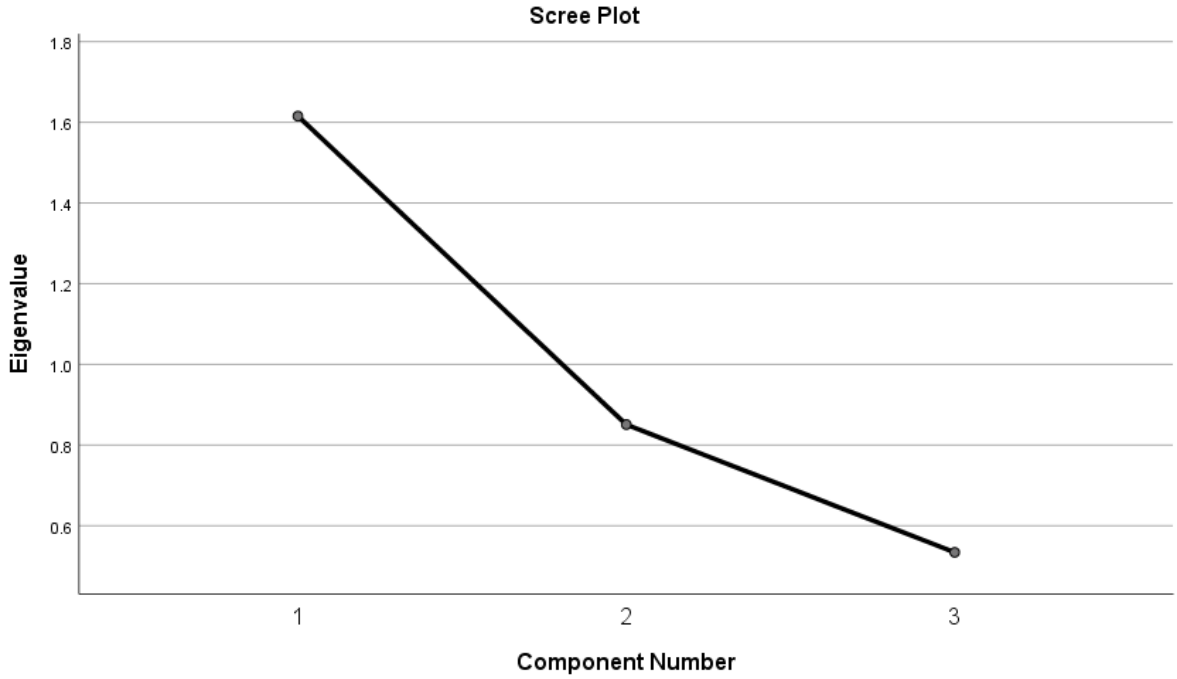
Component Matrix[a]	Component 1
ZStroop_Correct	0.79
ZPAB_Total_Errors	-0.46
ZCRT_Correct	0.75

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

Component	Initial Eigenvalues*				KMO and Bartlett's Test			
	N	Eigenvalue*	% of Variance	Cumulative %	KMO	Approx. Chi-Square	df	sig
Executive Function Speed	228	1.615	53.841	53.841	0.569	69.377	3	<0.001

*only eigenvalues ≥1 are reported



Component Matrix[a]	Component 1
ZStroop_Correct_RT	0.82
ZPAB_AVG_RT_Complete	0.58
ZCRT_Correct_RT	0.78

Extraction Method: Principal Component Analysis.

[a] 1 components extracted.

APPENDIX IV: Chapter 2 data

Predictors of iron status

Correlations

		Iron_status	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Iron_status	1.000	.119	.085	-.163	-.102	.015
	Age	.119	1.000	.255	-.140	-.037	.068
	BMI	.085	.255	1.000	-.068	.053	.021
	IPAQ	-.163	-.140	-.068	1.000	.105	.125
	MCQ	-.102	-.037	.053	.105	1.000	.078
	Iron	.015	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Iron_status	.	.035	.098	.007	.093	.412
	Age	.035	.	.000	.018	.317	.152
	BMI	.098	.000	.	.156	.248	.378
	IPAQ	.007	.018	.156	.	.091	.032
	MCQ	.093	.317	.248	.091	.	.157
	Iron	.412	.152	.378	.032	.157	.
N	Iron_status	235	235	235	225	170	230
	Age	235	235	235	225	170	230
	BMI	235	235	235	225	170	230
	IPAQ	225	225	225	225	163	221
	MCQ	170	170	170	163	170	167
	Iron	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Age[b]	.	Enter

a. Dependent Variable: Iron_status

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.217[a]	.047	.017	.460	.047	1.554	5	157	.176	.092

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

b. Dependent Variable: Iron_status

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.642	5	.328	1.554	.176[b]
	Residual	33.197	157	.211		
	Total	34.839	162			

a. Dependent Variable: Iron_status

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.515	.267		1.927	.056					
	Age	.004	.004	.078	.958	.339	.119	.076	.075	.912	1.097
	BMI	.007	.010	.059	.734	.464	.085	.058	.057	.930	1.076
	IPAQ	-3.154E-5	.000	-.142	-1.785	.076	-.163	-.141	-.139	.953	1.050
	MCQ	-.017	.015	-.090	-1.141	.256	-.102	-.091	-.089	.979	1.021
	Iron	.004	.010	.033	.418	.676	.015	.033	.033	.972	1.028

a. Dependent Variable: Iron_status

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Age
1	Correlations	Iron	1.000	-.003	-.068	-.129	-.085
		BMI	-.003	1.000	-.068	.040	-.249
		MCQ	-.068	-.068	1.000	-.093	.044
		IPAQ	-.129	.040	-.093	1.000	.132
		Age	-.085	-.249	.044	.132	1.000
	Covariances	Iron	.000	-3.220E-7	-1.067E-5	-2.336E-8	-3.782E-6
		BMI	-3.220E-7	9.293E-5	-9.974E-6	6.859E-9	-1.040E-5
		MCQ	-1.067E-5	-9.974E-6	.000	-2.527E-8	2.934E-6
		IPAQ	-2.336E-8	6.859E-9	-2.527E-8	3.121E-10	1.009E-8
		Age	-3.782E-6	-1.040E-5	2.934E-6	1.009E-8	1.877E-5

a. Dependent Variable: Iron_status

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Age	BMI	IPAQ	MCQ	Iron
1	1	5.294	1.000	.00	.00	.00	.01	.01	.00
	2	.288	4.286	.00	.01	.00	.12	.92	.01
	3	.272	4.413	.00	.05	.00	.75	.05	.01
	4	.086	7.825	.00	.25	.01	.05	.00	.79
	5	.048	10.524	.07	.70	.15	.04	.03	.15
	6	.011	21.610	.92	.00	.84	.03	.00	.05

a. Dependent Variable: Iron_status

Predictors of haemoglobin (SPSS output)

Correlations

		Haemoglobin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Haemoglobin	1.000	-.085	.127	-.021	-.002	.032
	Age	-.085	1.000	.255	-.140	-.037	.068
	BMI	.127	.255	1.000	-.068	.053	.021
	IPAQ	-.021	-.140	-.068	1.000	.105	.125
	MCQ	-.002	-.037	.053	.105	1.000	.078
	Iron	.032	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Haemoglobin	.	.098	.026	.374	.488	.312
	Age	.098	.	.000	.018	.317	.152
	BMI	.026	.000	.	.156	.248	.378
	IPAQ	.374	.018	.156	.	.091	.032
	MCQ	.488	.317	.248	.091	.	.157
	Iron	.312	.152	.378	.032	.157	.
N	Haemoglobin	235	235	235	225	170	230
	Age	235	235	235	225	170	230
	BMI	235	235	235	225	170	230
	IPAQ	225	225	225	225	163	221
	MCQ	170	170	170	163	170	167
	Iron	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Age[b]	.	Enter

a. Dependent Variable: Haemoglobin

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.184[a]	.034	.003	6.542	.034	1.097	5	157	.364	1.871

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

b. Dependent Variable: Haemoglobin

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	234.641	5	46.928	1.097	.364[b]
	Residual	6718.238	157	42.791		
	Total	6952.879	162			

a. Dependent Variable: Haemoglobin

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	123.425	3.803		32.452	.000					
	Age	-.100	.062	-.133	-1.625	.106	-.085	-.129	-.127	.912	1.097
	BMI	.268	.137	.159	1.955	.052	.127	.154	.153	.930	1.076
	IPAQ	.000	.000	-.033	-.413	.680	-.021	-.033	-.032	.953	1.050
	MCQ	-.043	.218	-.016	-.196	.845	-.002	-.016	-.015	.979	1.021
	Iron	.080	.146	.044	.549	.584	.032	.044	.043	.972	1.028

a. Dependent Variable: Haemoglobin

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Age
1	Correlations	Iron	1.000	-.003	-.068	-.129	-.085
		BMI	-.003	1.000	-.068	.040	-.249
		MCQ	-.068	-.068	1.000	-.093	.044
		IPAQ	-.129	.040	-.093	1.000	.132
		Age	-.085	-.249	.044	.132	1.000
	Covariances	Iron	.021	-6.516E-5	-.002	-4.727E-6	-.001
		BMI	-6.516E-5	.019	-.002	1.388E-6	-.002
		MCQ	-.002	-.002	.047	-5.115E-6	.001
		IPAQ	-4.727E-6	1.388E-6	-5.115E-6	6.316E-8	2.042E-6
		Age	-.001	-.002	.001	2.042E-6	.004

a. Dependent Variable: Haemoglobin

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Age	BMI	IPAQ	MCQ	Iron
1	1	5.294	1.000	.00	.00	.00	.01	.01	.00
	2	.288	4.286	.00	.01	.00	.12	.92	.01
	3	.272	4.413	.00	.05	.00	.75	.05	.01
	4	.086	7.825	.00	.25	.01	.05	.00	.79
	5	.048	10.524	.07	.70	.15	.04	.03	.15
	6	.011	21.610	.92	.00	.84	.03	.00	.05

a. Dependent Variable: Haemoglobin

Predictors of serum ferritin (SPSS output)

Correlations

		Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Ferritin	1.000	.181	.104	-.091	-.173	-.031
	Age	.181	1.000	.255	-.140	-.037	.068
	BMI	.104	.255	1.000	-.068	.053	.021
	IPAQ	-.091	-.140	-.068	1.000	.105	.125
	MCQ	-.173	-.037	.053	.105	1.000	.078
	Iron	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Ferritin	.	.003	.055	.087	.012	.322
	Age	.003	.	.000	.018	.317	.152
	BMI	.055	.000	.	.156	.248	.378
	IPAQ	.087	.018	.156	.	.091	.032
	MCQ	.012	.317	.248	.091	.	.157
	Iron	.322	.152	.378	.032	.157	.
N	Ferritin	235	235	235	225	170	230
	Age	235	235	235	225	170	230
	BMI	235	235	235	225	170	230
	IPAQ	225	225	225	225	163	221
	MCQ	170	170	170	163	170	167
	Iron	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Age[b]	.	Enter

a. Dependent Variable: Ferritin

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.261[a]	.068	.039	30.977	.068	2.301	5	157	.047	1.416

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

b. Dependent Variable: Ferritin

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11038.449	5	2207.690	2.301	.047[b]
	Residual	150650.207	157	959.555		
	Total	161688.656	162			

a. Dependent Variable: Ferritin

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Age

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	25.954	18.010		1.441	.152					
	Age	.551	.292	.152	1.887	.061	.181	.149	.145	.912	1.097
	BMI	.582	.649	.072	.896	.372	.104	.071	.069	.930	1.076
	IPAQ	-.001	.001	-.044	-.563	.574	-.091	-.045	-.043	.953	1.050
	MCQ	-2.181	1.032	-.165	-2.114	.036	-.173	-.166	-.163	.979	1.021
	Iron	-.212	.690	-.024	-.307	.759	-.031	-.025	-.024	.972	1.028

a. Dependent Variable: Ferritin

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Age
1	Correlations	Iron	1.000	-.003	-.068	-.129	-.085
		BMI	-.003	1.000	-.068	.040	-.249
		MCQ	-.068	-.068	1.000	-.093	.044
		IPAQ	-.129	.040	-.093	1.000	.132
		Age	-.085	-.249	.044	.132	1.000
	Covariances	Iron	.476	-.001	-.048	.000	-.017
		BMI	-.001	.422	-.045	3.113E-5	-.047
		MCQ	-.048	-.045	1.064	.000	.013
		IPAQ	.000	3.113E-5	.000	1.416E-6	4.579E-5
		Age	-.017	-.047	.013	4.579E-5	.085

a. Dependent Variable: Ferritin

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Age	BMI	IPAQ	MCQ	Iron
1	1	5.294	1.000	.00	.00	.00	.01	.01	.00
	2	.288	4.286	.00	.01	.00	.12	.92	.01
	3	.272	4.413	.00	.05	.00	.75	.05	.01
	4	.086	7.825	.00	.25	.01	.05	.00	.79
	5	.048	10.524	.07	.70	.15	.04	.03	.15
	6	.011	21.610	.92	.00	.84	.03	.00	.05

a. Dependent Variable: Ferritin

Predictors of cognitive function
Episodic Accuracy

Correlations

		Episodic_Accuracy	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Episodic_Accuracy	1.000	-.051	-.037	.100	-.104	-.060	.121	.029	.064
	Haemoglobin	-.051	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.037	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	.100	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	-.104	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	-.060	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	.121	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.029	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.064	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Episodic_Accuracy	.	.217	.287	.063	.056	.179	.035	.355	.167
	Haemoglobin	.217	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.287	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.063	.284	.033	.	.040	.244	.207	.391	.030
	Age	.056	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.179	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.035	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.355	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.167	.312	.322	.030	.152	.378	.032	.157	.
N	Episodic_Accuracy	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230

	Ferritin	235	235	235	234	235	235	225	170	230
	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Episodic_Accuracy

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.204[a]	.042	-.008	.71490	.042	.839	8	154	.570	1.624

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Episodic_Accuracy

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.429	8	.429	.839	.570[b]
	Residual	78.707	154	.511		
	Total	82.137	162			

a. Dependent Variable: Episodic_Accuracy

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

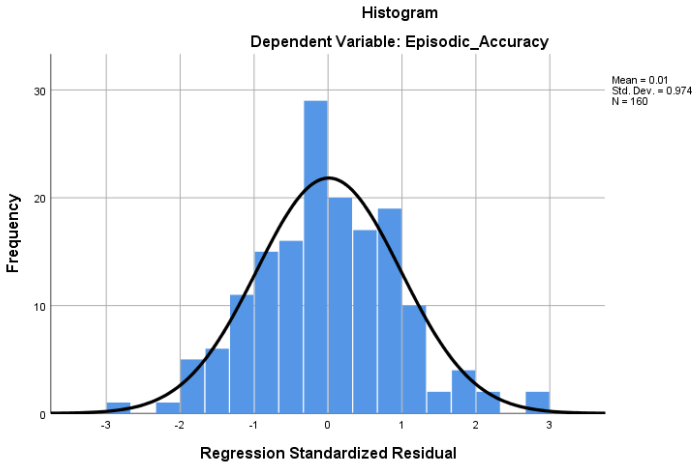
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.295	1.256		.235	.815					
	Haemoglobin	-.006	.009	-.051	-.605	.546	-.051	-.049	-.048	.889	1.125
	Ferritin	-6.738E-5	.002	-.003	-.035	.972	-.037	-.003	-.003	.849	1.178
	YIE	.032	.023	.112	1.384	.168	.100	.111	.109	.955	1.048
	Age	-.008	.007	-.102	-1.200	.232	-.104	-.096	-.095	.862	1.161
	BMI	-.005	.015	-.027	-.329	.743	-.060	-.026	-.026	.906	1.103
	IPAQ	3.473E-5	.000	.102	1.261	.209	.121	.101	.099	.948	1.055
	MCQ	.004	.024	.014	.172	.864	.029	.014	.014	.951	1.051
	Iron	.009	.016	.045	.560	.577	.064	.045	.044	.952	1.050

a. Dependent Variable: Episodic_Accuracy

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00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Dependent Variable: Episodic_Accuracy
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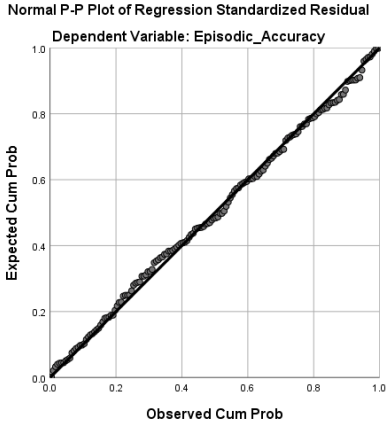
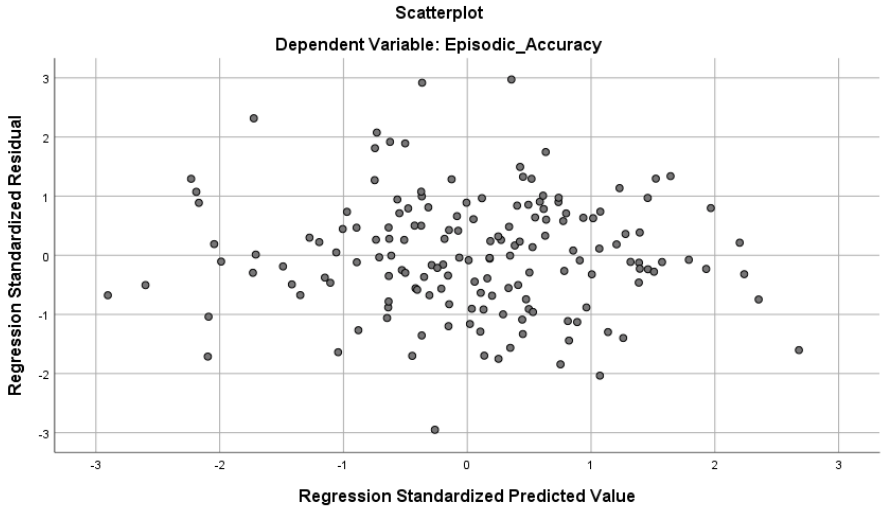
Std. Dev. = 0.974
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Episodic_Accuracy
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Episodic_Accuracy
Regression Standardized Residual
Regression Standardized Predicted Value

Episodic Speed

Correlations

		Episodic_Speed	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Episodic_Speed	1.000	-.018	-.067	-.007	.158	.080	-.037	-.024	.113
	Haemoglobin	-.018	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.067	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.007	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.158	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	.080	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	-.037	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	-.024	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.113	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Episodic_Speed	.	.393	.155	.455	.008	.113	.289	.377	.045
	Haemoglobin	.393	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.155	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.455	.284	.033	.	.040	.244	.207	.391	.030
	Age	.008	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.113	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.289	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.377	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.045	.312	.322	.030	.152	.378	.032	.157	.
N	Episodic_Speed	233	233	233	232	233	233	223	169	229
	Haemoglobin	233	235	235	234	235	235	225	170	230
	Ferritin	233	235	235	234	235	235	225	170	230

	YIE	232	234	234	234	234	234	224	169	229
	Age	233	235	235	234	235	235	225	170	230
	BMI	233	235	235	234	235	235	225	170	230
	IPAQ	223	225	225	224	225	225	225	163	221
	MCQ	169	170	170	169	170	170	163	170	167
	Iron	229	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Episodic_Speed

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.224[a]	.050	.001	.91521	.050	1.016	8	154	.426	2.099

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Episodic_Speed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.810	8	.851	1.016	.426[b]
	Residual	128.991	154	.838		
	Total	135.801	162			

a. Dependent Variable: Episodic_Speed

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

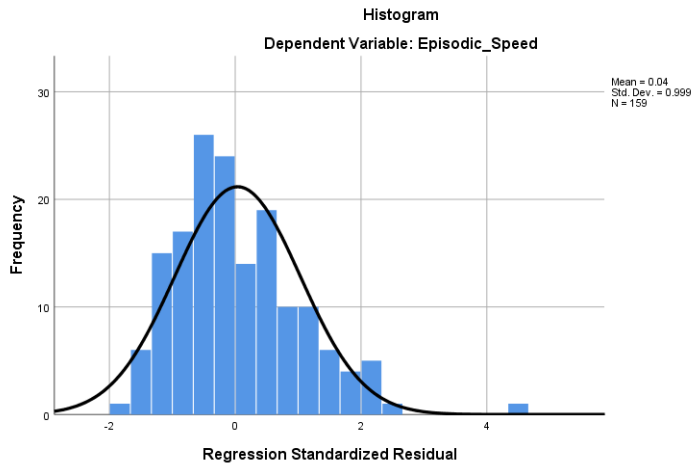
Coefficients[a]

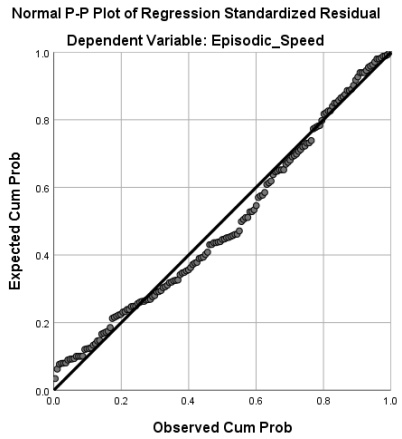
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.787	1.608		-.489	.625					
	Haemoglobin	.002	.012	.011	.133	.895	-.018	.011	.010	.889	1.125
	Ferritin	-.003	.002	-.107	-1.253	.212	-.067	-.100	-.098	.849	1.178
	YIE	-.011	.030	-.030	-.379	.705	-.007	-.031	-.030	.955	1.048
	Age	.016	.009	.155	1.835	.068	.158	.146	.144	.862	1.161
	BMI	.012	.019	.049	.598	.551	.080	.048	.047	.906	1.103
	IPAQ	-1.413E-5	.000	-.032	-.401	.689	-.037	-.032	-.031	.948	1.055
	MCQ	-.017	.031	-.045	-.563	.574	-.024	-.045	-.044	.951	1.051
	Iron	.028	.021	.109	1.352	.179	.113	.108	.106	.952	1.050

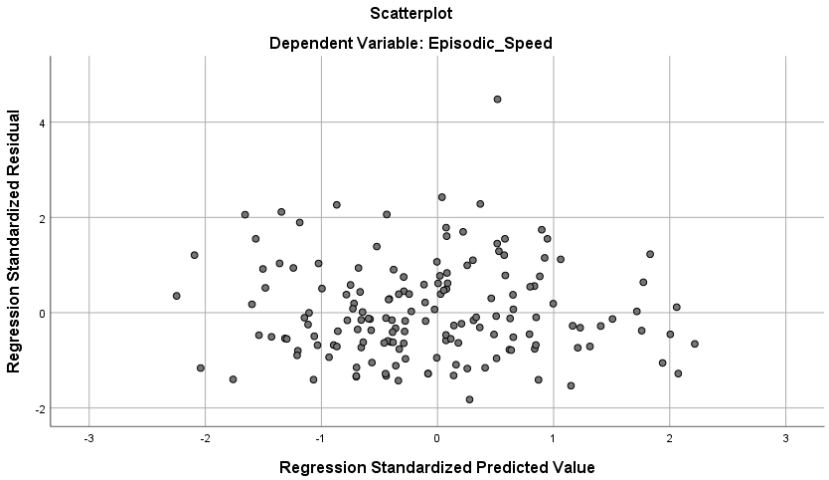
a. Dependent Variable: Episodic_Speed

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00






Scatterplot
Dependent Variable: Episodic_Speed
Regression Standardized Residual
Regression Standardized Predicted Value
4
2
0
-2
-3
-2
-1
0
1
2
3

Working Memory Accuracy

Correlations

		Working_Memory_Accuracy	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Working_Memory_Accuracy	1.000	.061	-.048	.184	.006	-.088	.087	.041	.059
	Haemoglobin	.061	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.048	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	.184	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.006	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	-.088	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	.087	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.041	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.059	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Working_Memory_Accuracy	.	.174	.231	.002	.462	.090	.098	.299	.187
	Haemoglobin	.174	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.231	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.002	.284	.033	.	.040	.244	.207	.391	.030
	Age	.462	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.090	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.098	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.299	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.187	.312	.322	.030	.152	.378	.032	.157	.

N	Working_Memory_Accuracy	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230
	Ferritin	235	235	235	234	235	235	225	170	230
	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Working_Memory_Accuracy

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.259[a]	.067	.019	.78956	.067	1.388	8	154	.206	1.765

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Working_Memory_Accuracy

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.920	8	.865	1.388	.206[b]
	Residual	96.005	154	.623		
	Total	102.925	162			

a. Dependent Variable: Working_Memory_Accuracy

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

Coefficients[a]

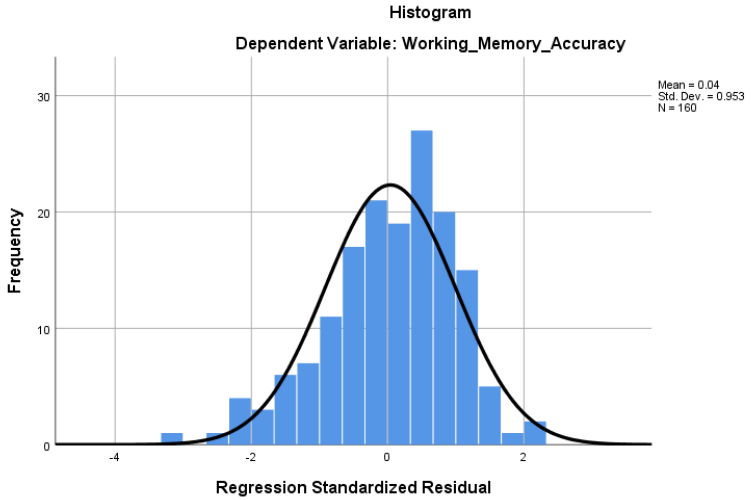
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-2.479	1.388		-1.787	.076					
	Haemoglobin	.013	.010	.111	1.339	.183	.061	.107	.104	.889	1.125
	Ferritin	-.002	.002	-.085	-1.007	.316	-.048	-.081	-.078	.849	1.178
	YIE	.065	.026	.201	2.525	.013	.184	.199	.196	.955	1.048

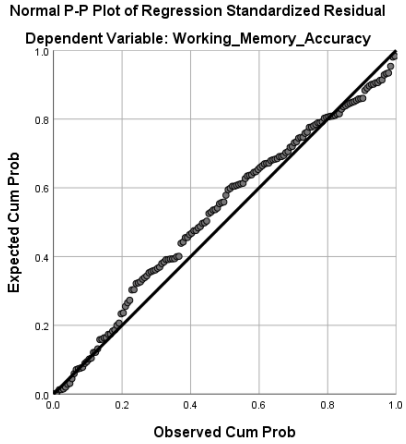
	Age	.004	.008	.048	.575	.566	.006	.046	.045	.862	1.161
	BMI	-.023	.017	-.110	-1.350	.179	-.088	-.108	-.105	.906	1.103
	IPAQ	3.305E-5	.000	.087	1.087	.279	.087	.087	.085	.948	1.055
	MCQ	.009	.027	.028	.350	.727	.041	.028	.027	.951	1.051
	Iron	.003	.018	.014	.171	.864	.059	.014	.013	.952	1.050

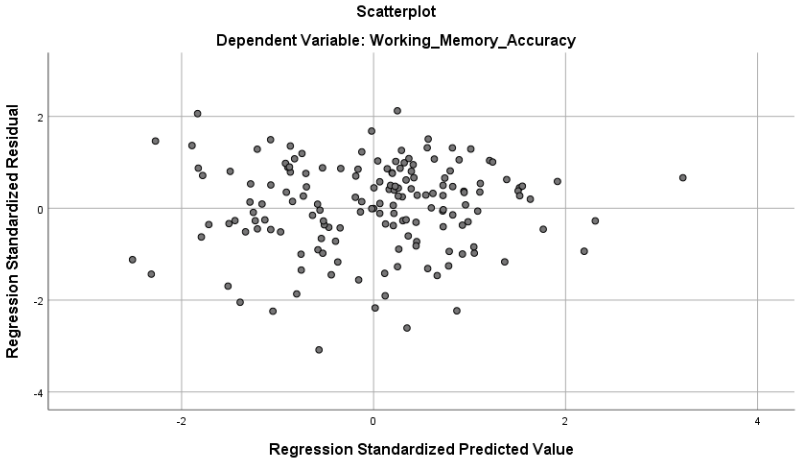
a. Dependent Variable: Working_Memory_Accuracy

Collinearity Diagnostics[a]

				Variance Proportions								
Model	Dimension	Eigenvalue	Condition Index	(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00	
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25	
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37	
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35	
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00	
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01	
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00	
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00	
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00	


Histogram
Dependent Variable: Working_Memory_Accuracy
Mean = 0.04
Std. Dev. = 0.953
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Working_Memory_Accuracy
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Working_Memory_Accuracy
Regression Standardized Residual
Regression Standardized Predicted Value

Working Memory Speed

Correlations

		Working_Memory_Speed	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Working_Memory_Speed	1.000	-.017	-.068	-.079	.097	-.041	-.013	.114	.087
	Haemoglobin	-.017	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.068	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.079	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.097	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	-.041	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	-.013	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.114	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.087	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Working_Memory_Speed	.	.401	.152	.117	.070	.267	.425	.072	.097
	Haemoglobin	.401	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.152	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.117	.284	.033	.	.040	.244	.207	.391	.030
	Age	.070	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.267	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.425	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.072	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.097	.312	.322	.030	.152	.378	.032	.157	.
N	Working_Memory_Speed	231	231	231	230	231	231	221	166	226
	Haemoglobin	231	235	235	234	235	235	225	170	230

	Ferritin	231	235	235	234	235	235	225	170	230
	YIE	230	234	234	234	234	234	224	169	229
	Age	231	235	235	234	235	235	225	170	230
	BMI	231	235	235	234	235	235	225	170	230
	IPAQ	221	225	225	224	225	225	225	163	221
	MCQ	166	170	170	169	170	170	163	170	167
	Iron	226	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Working_Memory_Speed

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.217[a]	.047	-.003	.99945	.047	.949	8	154	.478	1.932

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Working_Memory_Speed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7.584	8	.948	.949	.478[b]
	Residual	153.830	154	.999		
	Total	161.414	162			

a. Dependent Variable: Working_Memory_Speed

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

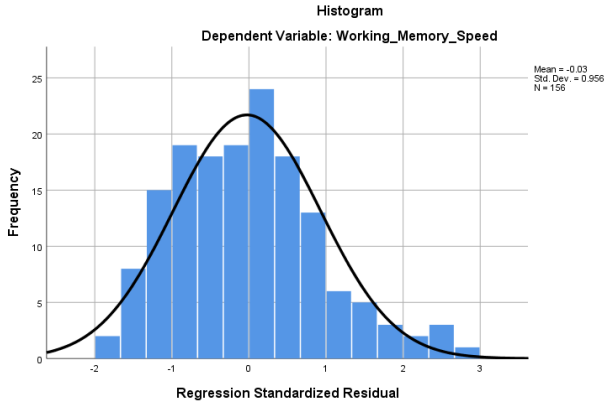
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.171	1.756		.097	.923					
	Haemoglobin	.002	.013	.013	.150	.881	-.017	.012	.012	.889	1.125
	Ferritin	-.002	.003	-.058	-.676	.500	-.068	-.054	-.053	.849	1.178
	YIE	-.038	.033	-.093	-1.156	.250	-.079	-.093	-.091	.955	1.048
	Age	.015	.010	.133	1.565	.120	.097	.125	.123	.862	1.161
	BMI	-.019	.021	-.076	-.917	.361	-.041	-.074	-.072	.906	1.103
	IPAQ	-1.489E-5	.000	-.031	-.387	.700	-.013	-.031	-.030	.948	1.055
	MCQ	.045	.034	.108	1.334	.184	.114	.107	.105	.951	1.051
	Iron	.023	.022	.084	1.041	.300	.087	.084	.082	.952	1.050

a. Dependent Variable: Working_Memory_Speed

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions								
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00	
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25	
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37	
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35	
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00	
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01	
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00	
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00	
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00	


Histogram
Dependent Variable: Working_Memory_Speed
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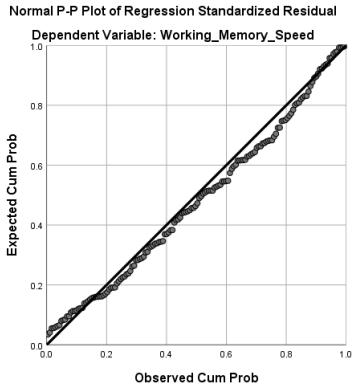
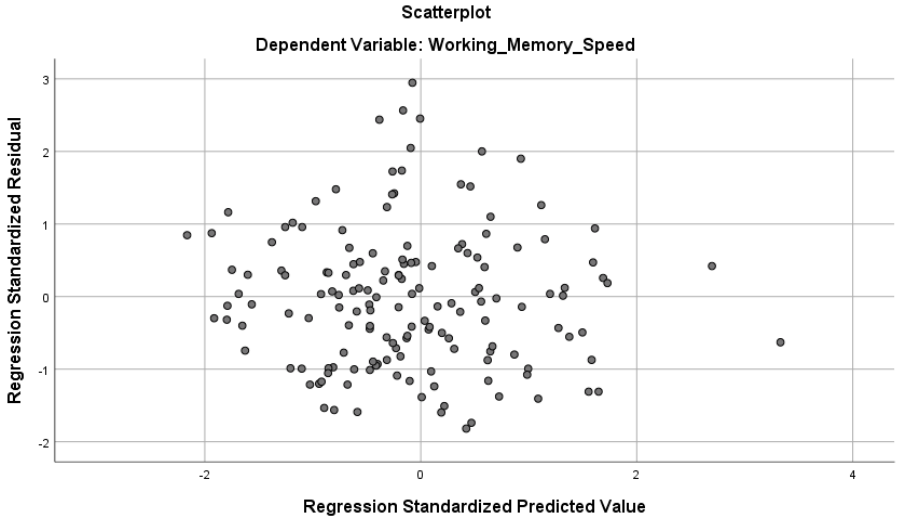
Std. Dev. = 0.956
N = 156
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Working_Memory_Speed
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Working_Memory_Speed
Regression Standardized Residual
Regression Standardized Predicted Value

Attention Accuracy

Correlations

		Attention_Accuracy	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Attention_Accuracy	1.000	.007	-.008	.136	.201	.017	.016	.005	-.037
	Haemoglobin	.007	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.008	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	.136	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.201	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	.017	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	.016	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.005	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	-.037	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Attention_Accuracy	.	.459	.453	.019	.001	.395	.404	.474	.289
	Haemoglobin	.459	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.453	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.019	.284	.033	.	.040	.244	.207	.391	.030
	Age	.001	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.395	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.404	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.474	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.289	.312	.322	.030	.152	.378	.032	.157	.
N	Attention_Accuracy	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230

	Ferritin	235	235	235	234	235	235	225	170	230
	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Attention_Accuracy

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.261[a]	.068	.020	.88784	.068	1.409	8	154	.197	1.825

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Attention_Accuracy

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	8.883	8	1.110	1.409	.197[b]
	Residual	121.393	154	.788		
	Total	130.276	162			

a. Dependent Variable: Attention_Accuracy

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

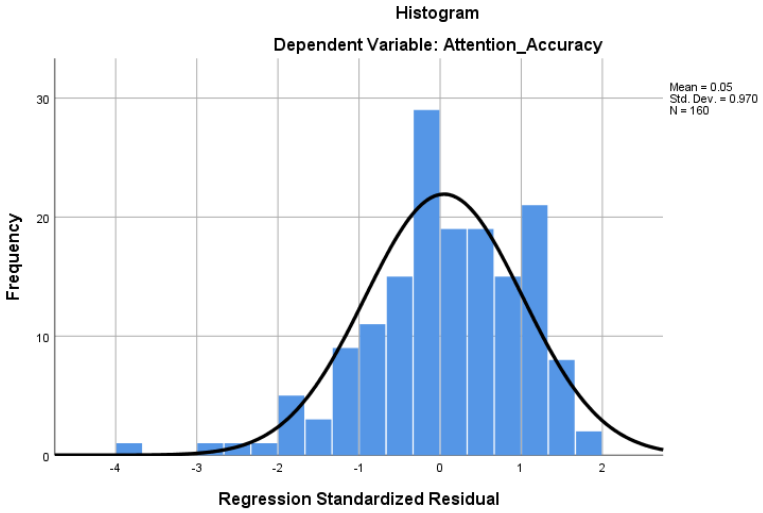
Coefficients[a]

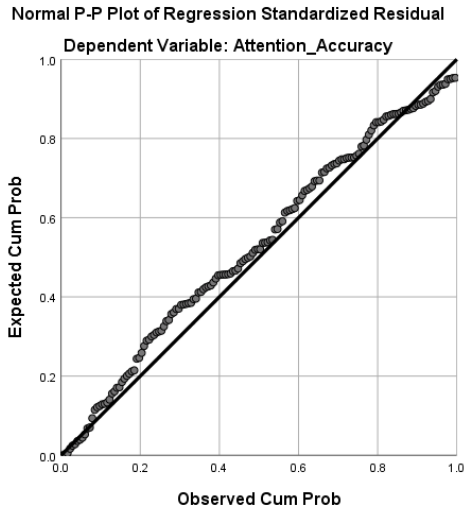
		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-2.086	1.560		-1.337	.183					
	Haemoglobin	.008	.011	.059	.713	.477	.007	.057	.055	.889	1.125
	Ferritin	-.002	.002	-.072	-.857	.393	-.008	-.069	-.067	.849	1.178
	YIE	.049	.029	.136	1.705	.090	.136	.136	.133	.955	1.048
	Age	.023	.009	.227	2.713	.007	.201	.214	.211	.862	1.161
	BMI	-.010	.019	-.041	-.507	.613	.017	-.041	-.039	.906	1.103
	IPAQ	2.430E-5	.000	.057	.711	.478	.016	.057	.055	.948	1.055
	MCQ	.002	.030	.006	.080	.937	.005	.006	.006	.951	1.051
	Iron	-.020	.020	-.080	-1.006	.316	-.037	-.081	-.078	.952	1.050

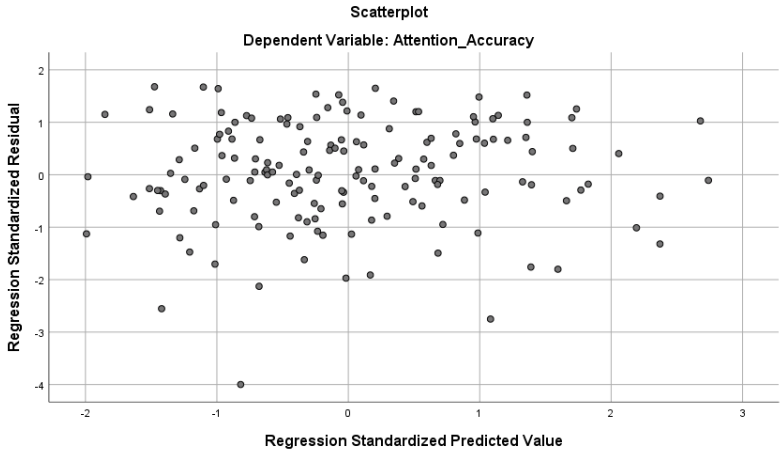
a. Dependent Variable: Attention_Accuracy

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions								
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00	
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25	
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37	
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35	
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00	
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01	
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00	
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00	
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00	


Histogram
Dependent Variable: Attention_Accuracy
Mean = 0.05
Std. Dev. = 0.970
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Attention_Accuracy
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Attention_Accuracy
Regression Standardized Residual
Regression Standardized Predicted Value

Attention Speed

Correlations

		Attention_Speed	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Attention_Speed	1.000	.020	-.012	-.089	-.027	-.034	.069	-.023	.100
	Haemoglobin	.020	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.012	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.089	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	-.027	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	-.034	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	.069	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	-.023	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.100	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Attention_Speed	.	.379	.425	.088	.340	.301	.150	.385	.066
	Haemoglobin	.379	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.425	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.088	.284	.033	.	.040	.244	.207	.391	.030
	Age	.340	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.301	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.150	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.385	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.066	.312	.322	.030	.152	.378	.032	.157	.
N	Attention_Speed	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230
	Ferritin	235	235	235	234	235	235	225	170	230

	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Attention_Speed

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.160[a]	.025	-.025	.86163	.025	.503	8	154	.853	1.839

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Attention_Speed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.986	8	.373	.503	.853[b]
	Residual	114.329	154	.742		
	Total	117.315	162			

a. Dependent Variable: Attention_Speed

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

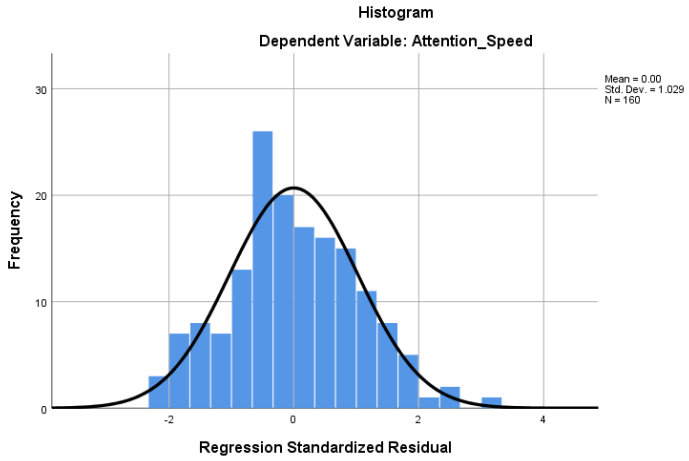
Coefficients[a]

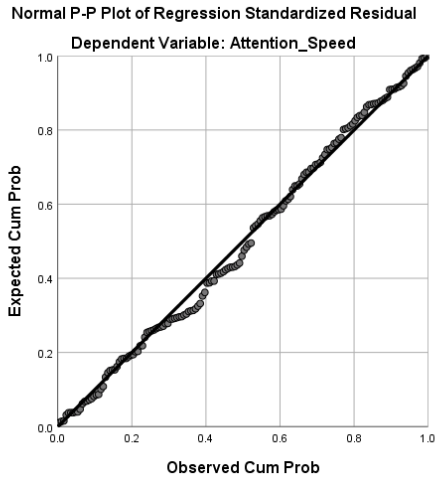
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.191	1.514		.126	.900					
	Haemoglobin	.002	.011	.016	.193	.847	.020	.016	.015	.889	1.125
	Ferritin	3.276E-5	.002	.001	.014	.989	-.012	.001	.001	.849	1.178
	YIE	-.034	.028	-.098	-1.199	.233	-.089	-.096	-.095	.955	1.048
	Age	-.001	.008	-.009	-.111	.912	-.027	-.009	-.009	.862	1.161
	BMI	-.006	.018	-.026	-.316	.753	-.034	-.025	-.025	.906	1.103
	IPAQ	2.101E-5	.000	.052	.633	.528	.069	.051	.050	.948	1.055
	MCQ	-.013	.029	-.037	-.458	.648	-.023	-.037	-.036	.951	1.051
	Iron	.026	.019	.109	1.337	.183	.100	.107	.106	.952	1.050

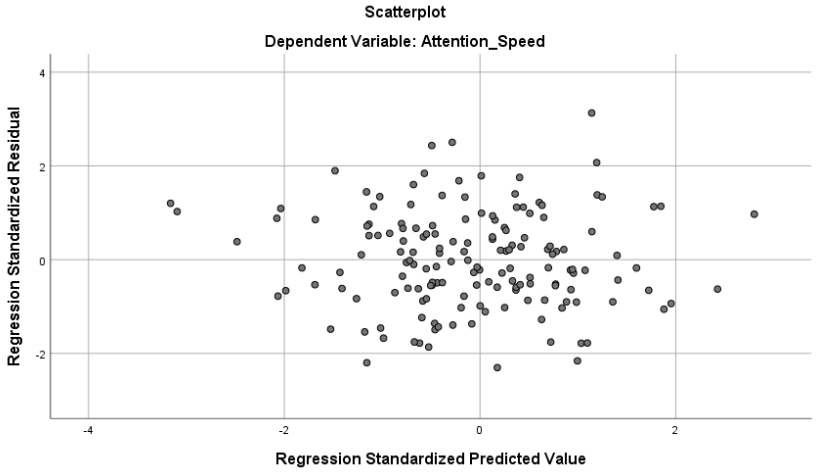
a. Dependent Variable: Attention_Speed

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions								
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00	
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25	
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37	
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35	
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00	
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01	
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00	
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00	
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00	


Histogram
Dependent Variable: Attention_Speed
Mean = 0.00
Std. Dev. = 1.029
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Attention_Speed
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Attention_Speed
Regression Standardized Residual
Regression Standardized Predicted Value

Learning (CLLT)

Correlations

		CLLT	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	CLLT	1.000	.054	-.033	.055	-.138	-.182	.042	.070	-.036
	Haemoglobin	.054	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.033	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	.055	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	-.138	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	-.182	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	.042	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.070	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	-.036	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	CLLT	.	.207	.310	.202	.018	.003	.269	.186	.296
	Haemoglobin	.207	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.310	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.202	.284	.033	.	.040	.244	.207	.391	.030
	Age	.018	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.003	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.269	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.186	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.296	.312	.322	.030	.152	.378	.032	.157	.
N	CLLT	230	230	230	229	230	230	220	167	225
	Haemoglobin	230	235	235	234	235	235	225	170	230
	Ferritin	230	235	235	234	235	235	225	170	230
	YIE	229	234	234	234	234	234	224	169	229

	Age	230	235	235	234	235	235	225	170	230
	BMI	230	235	235	234	235	235	225	170	230
	IPAQ	220	225	225	224	225	225	225	163	221
	MCQ	167	170	170	169	170	170	163	170	167
	Iron	225	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: CLLT

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.247[a]	.061	.012	.75300	.061	1.249	8	154	.274	2.000

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: CLLT

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.667	8	.708	1.249	.274[b]
	Residual	87.318	154	.567		
	Total	92.985	162			

a. Dependent Variable: CLLT

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

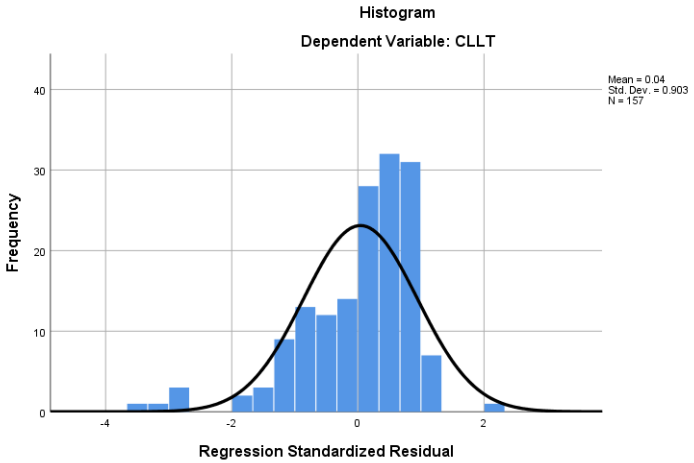
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.558	1.323		-.422	.674					
	Haemoglobin	.009	.010	.079	.952	.343	.054	.076	.074	.889	1.125
	Ferritin	.000	.002	-.016	-.194	.846	-.033	-.016	-.015	.849	1.178
	YIE	.027	.025	.087	1.088	.278	.055	.087	.085	.955	1.048
	Age	-.007	.007	-.085	-1.012	.313	-.138	-.081	-.079	.862	1.161
	BMI	-.034	.016	-.174	-2.119	.036	-.182	-.168	-.165	.906	1.103
	IPAQ	7.618E-6	.000	.021	.263	.793	.042	.021	.021	.948	1.055
	MCQ	.024	.025	.077	.956	.341	.070	.077	.075	.951	1.051
	Iron	-.010	.017	-.049	-.612	.541	-.036	-.049	-.048	.952	1.050

a. Dependent Variable: CLLT

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00


Histogram
Dependent Variable: CLLT
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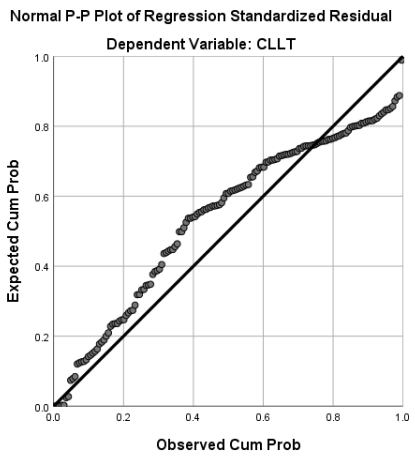
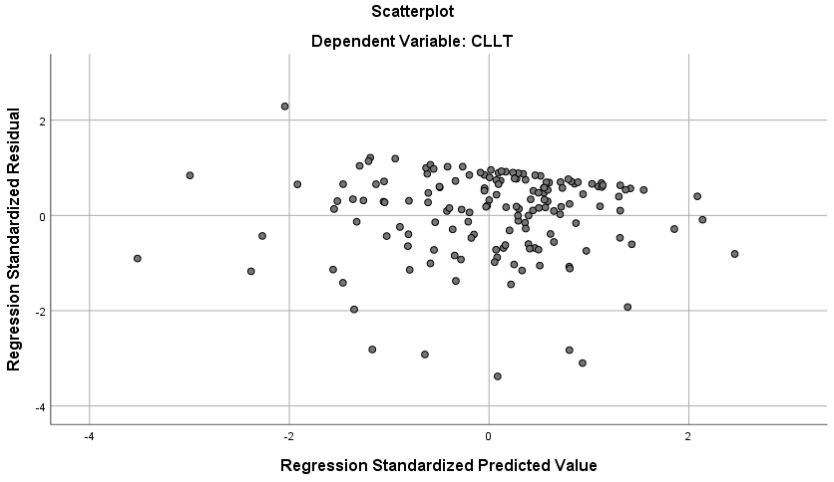
Std. Dev. = 0.903
N = 157
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: CLLT
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: CLLT
Regression Standardized Residual
Regression Standardized Predicted Value

Executive Function Accuracy

Correlations

		Executive_Function_Accuracy	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Executive_Function_Accuracy	1.000	.098	.019	-.022	-.037	.002	-.059	.063	.014
	Haemoglobin	.098	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	.019	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.022	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	-.037	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	.002	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	-.059	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.063	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.014	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Executive_Function_Accuracy	.	.067	.385	.367	.287	.489	.187	.208	.418
	Haemoglobin	.067	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.385	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.367	.284	.033	.	.040	.244	.207	.391	.030
	Age	.287	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.489	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.187	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.208	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.418	.312	.322	.030	.152	.378	.032	.157	.

N	Executive_Function_Accuracy	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230
	Ferritin	235	235	235	234	235	235	225	170	230
	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Executive_Function_Accuracy

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.141[a]	.020	-.031	.68017	.020	.391	8	154	.924	1.966

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Executive_Function_Accuracy

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.446	8	.181	.391	.924[b]
	Residual	71.244	154	.463		
	Total	72.690	162			

a. Dependent Variable: Executive_Function_Accuracy

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

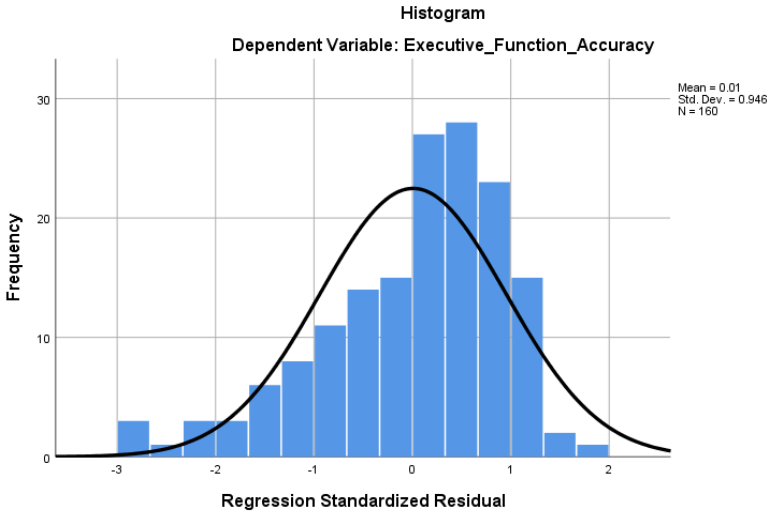
Coefficients[a]

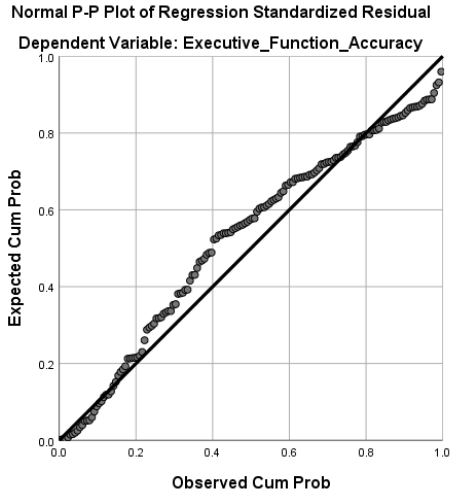
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.015	1.195		-.849	.397					
	Haemoglobin	.009	.009	.091	1.071	.286	.098	.086	.085	.889	1.125
	Ferritin	.000	.002	.012	.136	.892	.019	.011	.011	.849	1.178
	YIE	-.006	.022	-.021	-.254	.800	-.022	-.020	-.020	.955	1.048
	Age	-.003	.007	-.035	-.413	.680	-.037	-.033	-.033	.862	1.161
	BMI	-.002	.014	-.010	-.119	.906	.002	-.010	-.009	.906	1.103
	IPAQ	-2.337E-5	.000	-.073	-.892	.374	-.059	-.072	-.071	.948	1.055
	MCQ	.020	.023	.070	.855	.394	.063	.069	.068	.951	1.051
	Iron	.004	.015	.020	.244	.807	.014	.020	.020	.952	1.050

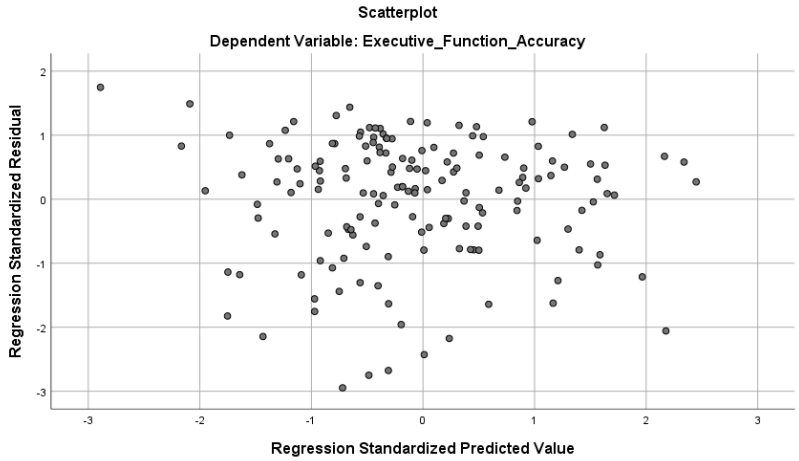
a. Dependent Variable: Executive_Function_Accuracy

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00


Histogram
Dependent Variable: Executive_Function_Accuracy
Mean = 0.01
Std. Dev. = 0.946
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Executive_Function_Accuracy
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Executive_Function_Accuracy
Regression Standardized Residual
Regression Standardized Predicted Value

Executive Function Speed

Correlations

		Executive_Function_Speed	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Executive_Function_Speed	1.000	.037	.050	-.026	.247	.141	-.011	.048	.072
	Haemoglobin	.037	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	.050	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.026	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.247	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	.141	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	-.011	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.048	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.072	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Executive_Function_Speed	.	.284	.223	.346	.000	.015	.437	.265	.140
	Haemoglobin	.284	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.223	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.346	.284	.033	.	.040	.244	.207	.391	.030
	Age	.000	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.015	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.437	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.265	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.140	.312	.322	.030	.152	.378	.032	.157	.
N	Executive_Function_Speed	235	235	235	234	235	235	225	170	230
	Haemoglobin	235	235	235	234	235	235	225	170	230

	Ferritin	235	235	235	234	235	235	225	170	230
	YIE	234	234	234	234	234	234	224	169	229
	Age	235	235	235	234	235	235	225	170	230
	BMI	235	235	235	234	235	235	225	170	230
	IPAQ	225	225	225	224	225	225	225	163	221
	MCQ	170	170	170	169	170	170	163	170	167
	Iron	230	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Executive_Function_Speed

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.280[a]	.079	.031	.72139	.079	1.642	8	154	.117	1.877

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Executive_Function_Speed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.837	8	.855	1.642	.117[b]
	Residual	80.142	154	.520		
	Total	86.980	162			

a. Dependent Variable: Executive_Function_Speed

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

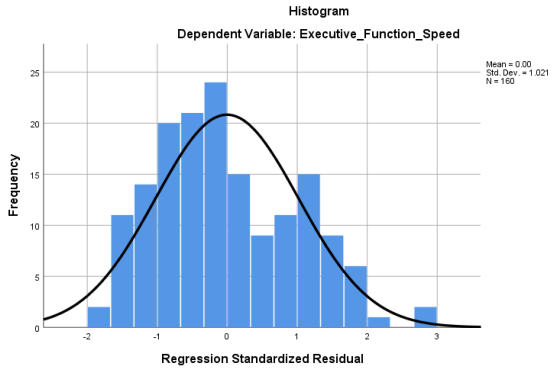
Coefficients[a]

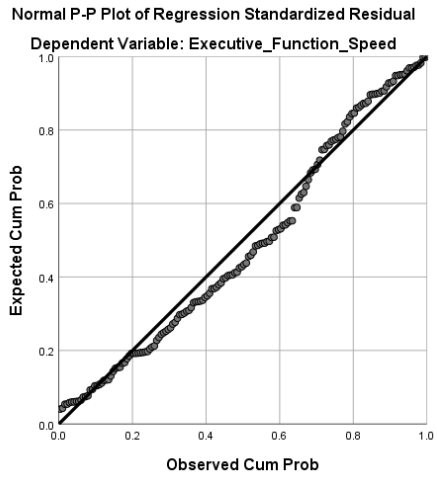
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.385	1.268		-1.092	.276					
	Haemoglobin	.005	.009	.043	.519	.604	.037	.042	.040	.889	1.125
	Ferritin	.000	.002	.007	.080	.936	.050	.006	.006	.849	1.178
	YIE	-.018	.023	-.061	-.771	.442	-.026	-.062	-.060	.955	1.048
	Age	.020	.007	.237	2.843	.005	.247	.223	.220	.862	1.161
	BMI	.014	.015	.075	.921	.358	.141	.074	.071	.906	1.103
	IPAQ	4.856E-6	.000	.014	.175	.861	-.011	.014	.014	.948	1.055
	MCQ	.015	.024	.047	.597	.551	.048	.048	.046	.951	1.051
	Iron	.011	.016	.055	.693	.490	.072	.056	.054	.952	1.050

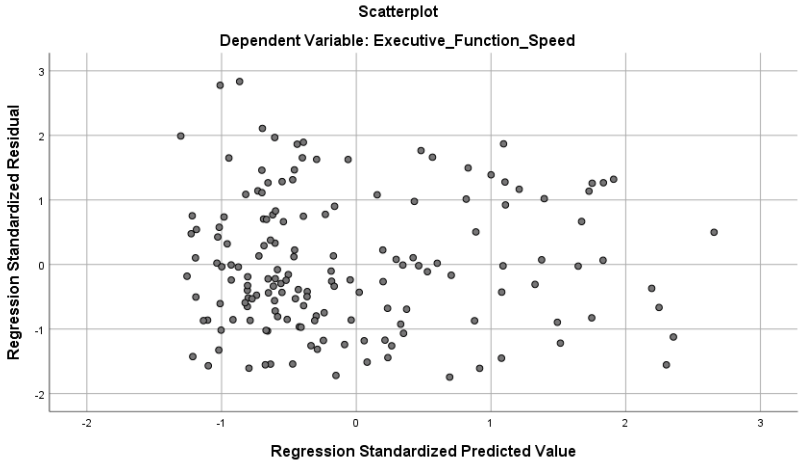
a. Dependent Variable: Executive_Function_Speed

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00


Histogram
Dependent Variable: Executive_Function_Speed
Mean = 0.00
Std. Dev. = 1.021
N = 160
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Executive_Function_Speed
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: Executive_Function_Speed
Regression Standardized Residual
Regression Standardized Predicted Value

Predictors of subjective mood (POMS)
Tension-Anxiety

Correlations

		Tension-Anxiety	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Tension-Anxiety	1.000	.088	.000	-.071	-.053	.080	.024	-.019
	Haemoglobin	.088	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	.000	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.071	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.053	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.080	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	.024	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	-.019	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Tension-Anxiety	.	.091	.500	.142	.210	.118	.379	.387
	Haemoglobin	.091	.	.000	.126	.016	.431	.425	.211
	Ferritin	.500	.000	.	.001	.060	.132	.014	.371
	Age	.142	.126	.001	.	.000	.012	.323	.226
	BMI	.210	.016	.060	.000	.	.152	.263	.376
	IPAQ	.118	.431	.132	.012	.152	.	.167	.071
	MCQ	.379	.425	.014	.323	.263	.167	.	.283
	Iron	.387	.211	.371	.226	.376	.071	.283	.
N	Tension-Anxiety	230	230	230	230	230	220	167	225
	Haemoglobin	230	231	231	231	231	221	168	226
	Ferritin	230	231	231	231	231	221	168	226
	Age	230	231	231	231	231	221	168	226

	BMI	230	231	231	231	231	221	168	226
	IPAQ	220	221	221	221	221	221	161	217
	MCQ	167	168	168	168	168	161	168	165
	Iron	225	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Tension-Anxiety

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.144[a]	.021	-.024	4.888	.021	.464	7	153	.860	2.046

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Tension-Anxiety

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	77.546	7	11.078	.464	.860[b]
	Residual	3654.920	153	23.888		
	Total	3732.466	160			

a. Dependent Variable: Tension-Anxiety

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.784	8.150		-.096	.924					
	Haemoglobin	.072	.063	.097	1.135	.258	.088	.091	.091	.878	1.138
	Ferritin	-.001	.013	-.006	-.068	.946	.000	-.006	-.005	.848	1.179
	Age	-.020	.049	-.035	-.405	.686	-.071	-.033	-.032	.851	1.175
	BMI	-.065	.105	-.052	-.617	.538	-.053	-.050	-.049	.896	1.116
	IPAQ	.000	.000	.074	.902	.369	.080	.073	.072	.959	1.043
	MCQ	.038	.167	.019	.228	.820	.024	.018	.018	.958	1.043
	Iron	-.041	.110	-.030	-.370	.712	-.019	-.030	-.030	.979	1.022

a. Dependent Variable: Tension-Anxiety

Coefficient Correlations[a]

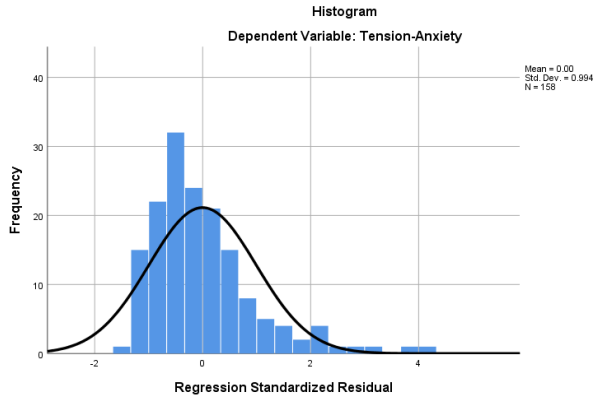
Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.012	1.147E-5	-.001	-2.221E-6	.000	.000	6.113E-5
		BMI	1.147E-5	.011	-.001	5.995E-7	-.001	-.001	-1.767E-5
		MCQ	-.001	-.001	.028	-2.029E-6	-.001	3.718E-5	.000
		IPAQ	-2.221E-6	5.995E-7	-2.029E-6	3.581E-8	2.000E-7	1.228E-6	6.286E-8
		Haemoglobin	.000	-.001	-.001	2.000E-7	.004	.001	.000
		Age	.000	-.001	3.718E-5	1.228E-6	.001	.002	.000
		Ferritin	6.113E-5	-1.767E-5	.000	6.286E-8	.000	.000	.000

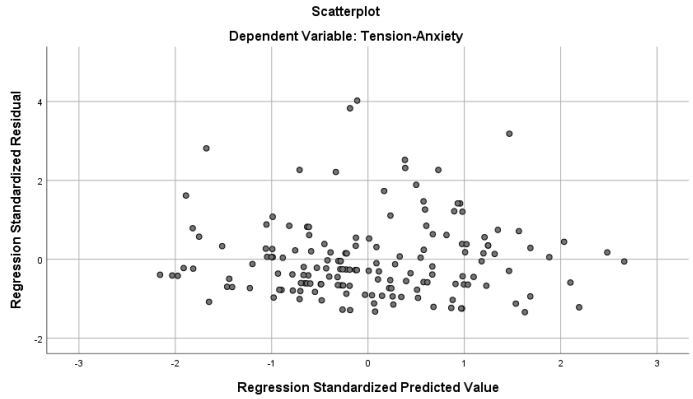
a. Dependent Variable: Tension-Anxiety

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.129	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.859	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.288	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.468	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.200	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Tension-Anxiety





Depression-Dejection

Correlations

		Depression-Dejection	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Depression-Dejection	1.000	-.023	.023	-.055	-.143	.042	-.004	.040
	Haemoglobin	-.023	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	.023	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.055	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.143	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.042	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	-.004	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	.040	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Depression-Dejection	.	.366	.365	.209	.017	.270	.480	.279
	Haemoglobin	.366	.	.000	.126	.016	.431	.425	.211
	Ferritin	.365	.000	.	.001	.060	.132	.014	.371
	Age	.209	.126	.001	.	.000	.012	.323	.226
	BMI	.017	.016	.060	.000	.	.152	.263	.376
	IPAQ	.270	.431	.132	.012	.152	.	.167	.071
	MCQ	.480	.425	.014	.323	.263	.167	.	.283
	Iron	.279	.211	.371	.226	.376	.071	.283	.
N	Depression-Dejection	219	219	219	219	219	211	160	216
	Haemoglobin	219	231	231	231	231	221	168	226
	Ferritin	219	231	231	231	231	221	168	226
	Age	219	231	231	231	231	221	168	226

	BMI	219	231	231	231	231	221	168	226
	IPAQ	211	221	221	221	221	221	161	217
	MCQ	160	168	168	168	168	161	168	165
	Iron	216	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Depression-Dejection

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.160[a]	.026	-.019	2.961	.026	.574	7	152	.777	1.859

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Depression-Dejection

ANOVA[a]

Model	Sum of Squares	df	Mean Square	F	Sig.

1	Regression	35.208	7	5.030	.574	.777[b]
	Residual	1333.014	152	8.770		
	Total	1368.222	159			

a. Dependent Variable: Depression-Dejection

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	5.316	4.953		1.073	.285					
	Haemoglobin	-.010	.038	-.023	-.265	.791	-.023	-.022	-.021	.878	1.138
	Ferritin	.005	.008	.053	.614	.540	.023	.050	.049	.848	1.179
	Age	-.010	.030	-.028	-.323	.747	-.055	-.026	-.026	.851	1.175
	BMI	-.103	.064	-.137	-1.617	.108	-.143	-.130	-.129	.896	1.116
	IPAQ	3.881E-5	.000	.028	.337	.736	.042	.027	.027	.959	1.043
	MCQ	.009	.102	.007	.086	.932	-.004	.007	.007	.958	1.043
	Iron	.036	.067	.044	.539	.591	.040	.044	.043	.979	1.022

a. Dependent Variable: Depression-Dejection

Coefficient Correlations[a]

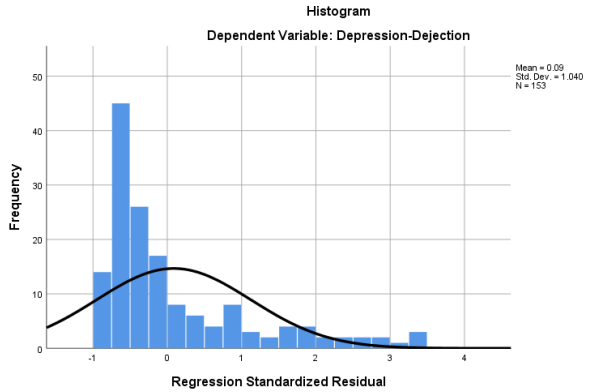
Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.004	4.238E-6	.000	-8.204E-7	.000	.000	2.258E-5
		BMI	4.238E-6	.004	.000	2.215E-7	.000	-.001	-6.529E-6
		MCQ	.000	.000	.010	-7.497E-7	.000	1.374E-5	.000
		IPAQ	-8.204E-7	2.215E-7	-7.497E-7	1.323E-8	7.390E-8	4.535E-7	2.322E-8
		Haemoglobin	.000	.000	.000	7.390E-8	.001	.000	-9.210E-5
		Age	.000	-.001	1.374E-5	4.535E-7	.000	.001	-5.028E-5
		Ferritin	2.258E-5	-6.529E-6	.000	2.322E-8	-9.210E-5	-5.028E-5	6.617E-5

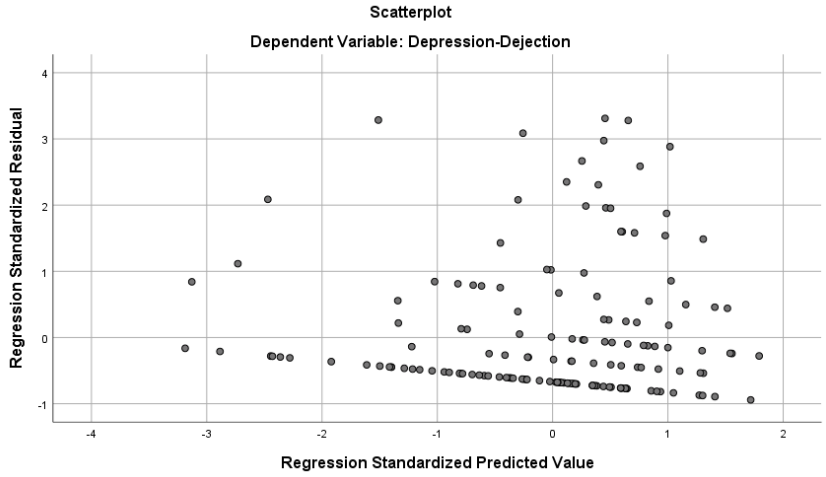
a. Dependent Variable: Depression-Dejection

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.129	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.859	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.288	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.469	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.202	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Depression-Dejection





Anger-Hostility

Correlations

		Anger-Hostility	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Anger-Hostility	1.000	.015	-.002	-.037	-.078	.130	-.006	-.012
	Haemoglobin	.015	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	-.002	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.037	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.078	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.130	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	-.006	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	-.012	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Anger-Hostility	.	.411	.486	.294	.125	.030	.470	.428
	Haemoglobin	.411	.	.000	.126	.016	.431	.425	.211
	Ferritin	.486	.000	.	.001	.060	.132	.014	.371
	Age	.294	.126	.001	.	.000	.012	.323	.226
	BMI	.125	.016	.060	.000	.	.152	.263	.376
	IPAQ	.030	.431	.132	.012	.152	.	.167	.071
	MCQ	.470	.425	.014	.323	.263	.167	.	.283
	Iron	.428	.211	.371	.226	.376	.071	.283	.
N	Anger-Hostility	218	218	218	218	218	209	158	215
	Haemoglobin	218	231	231	231	231	221	168	226
	Ferritin	218	231	231	231	231	221	168	226
	Age	218	231	231	231	231	221	168	226
	BMI	218	231	231	231	231	221	168	226

	IPAQ	209	221	221	221	221	221	161	217
	MCQ	158	168	168	168	168	161	168	165
	Iron	215	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Anger-Hostility

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.153[a]	.023	-.022	2.027	.023	.512	7	150	.824	1.962

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Anger-Hostility

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	14.734	7	2.105	.512	.824[b]
	Residual	616.508	150	4.110		
	Total	631.242	157			

a. Dependent Variable: Anger-Hostility

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	1.069	3.413		.313	.754					
	Haemoglobin	.009	.026	.028	.327	.744	.015	.027	.026	.878	1.138
	Ferritin	.000	.006	.004	.045	.964	-.002	.004	.004	.848	1.179
	Age	.001	.020	.005	.057	.955	-.037	.005	.005	.851	1.175
	BMI	-.038	.044	-.074	-.868	.387	-.078	-.071	-.070	.896	1.116
	IPAQ	.000	.000	.130	1.576	.117	.130	.128	.127	.959	1.043
	MCQ	-.009	.070	-.011	-.132	.895	-.006	-.011	-.011	.958	1.043
	Iron	-.014	.046	-.025	-.307	.759	-.012	-.025	-.025	.979	1.022

a. Dependent Variable: Anger-Hostility

Coefficient Correlations[a]

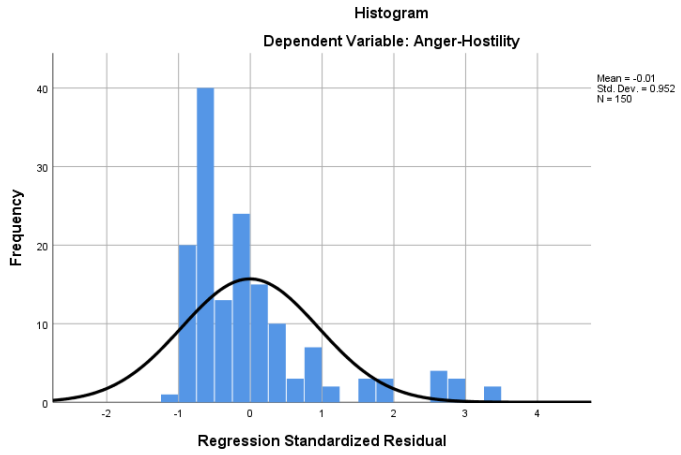
Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.002	2.011E-6	-9.935E-5	-3.894E-7	-8.432E-5	-7.200E-5	1.072E-5
		BMI	2.011E-6	.002	.000	1.051E-7	.000	.000	-3.099E-6
		MCQ	-9.935E-5	.000	.005	-3.558E-7	-9.621E-5	6.519E-6	6.803E-5
		IPAQ	-3.894E-7	1.051E-7	-3.558E-7	6.279E-9	3.508E-8	2.152E-7	1.102E-8
		Haemoglobin	-8.432E-5	.000	-9.621E-5	3.508E-8	.001	9.580E-5	-4.371E-5
		Age	-7.200E-5	.000	6.519E-6	2.152E-7	9.580E-5	.000	-2.386E-5
		Ferritin	1.072E-5	-3.099E-6	6.803E-5	1.102E-8	-4.371E-5	-2.386E-5	3.141E-5

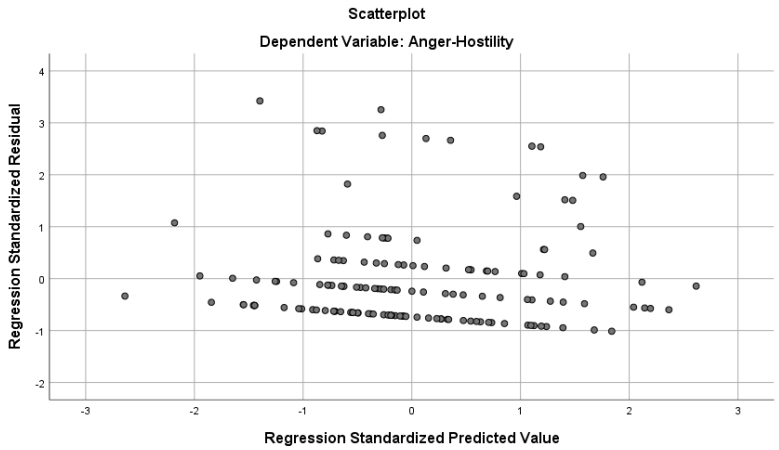
a. Dependent Variable: Anger-Hostility

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.130	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.860	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.289	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.470	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.205	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Anger-Hostility





Vigour-Activity

Correlations

		Vigour-Activity	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Vigour-Activity	1.000	-.002	.095	.138	.024	.083	.043	.096
	Haemoglobin	-.002	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	.095	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	.138	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	.024	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.083	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	.043	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	.096	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Vigour-Activity	.	.485	.075	.018	.357	.110	.288	.075
	Haemoglobin	.485	.	.000	.126	.016	.431	.425	.211
	Ferritin	.075	.000	.	.001	.060	.132	.014	.371
	Age	.018	.126	.001	.	.000	.012	.323	.226
	BMI	.357	.016	.060	.000	.	.152	.263	.376
	IPAQ	.110	.431	.132	.012	.152	.	.167	.071
	MCQ	.288	.425	.014	.323	.263	.167	.	.283
	Iron	.075	.211	.371	.226	.376	.071	.283	.
N	Vigour-Activity	231	231	231	231	231	221	168	226
	Haemoglobin	231	231	231	231	231	221	168	226
	Ferritin	231	231	231	231	231	221	168	226
	Age	231	231	231	231	231	221	168	226
	BMI	231	231	231	231	231	221	168	226
	IPAQ	221	221	221	221	221	221	161	217

	MCQ	168	168	168	168	168	161	168	165
	Iron	226	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Vigour-Activity

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.213[a]	.045	.002	6.274	.045	1.038	7	153	.407	1.797

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Vigour-Activity

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	286.111	7	40.873	1.038	.407[b]
	Residual	6022.081	153	39.360		
	Total	6308.192	160			

a. Dependent Variable: Vigour-Activity

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	9.493	10.461		.907	.366					
	Haemoglobin	-.018	.081	-.019	-.225	.822	-.002	-.018	-.018	.878	1.138
	Ferritin	.019	.017	.093	1.086	.279	.095	.087	.086	.848	1.179
	Age	.099	.062	.136	1.586	.115	.138	.127	.125	.851	1.175
	BMI	-.028	.135	-.017	-.205	.838	.024	-.017	-.016	.896	1.116
	IPAQ	.000	.000	.097	1.200	.232	.083	.097	.095	.959	1.043
	MCQ	.144	.215	.054	.670	.504	.043	.054	.053	.958	1.043
	Iron	.142	.141	.080	1.007	.315	.096	.081	.080	.979	1.022

a. Dependent Variable: Vigour-Activity

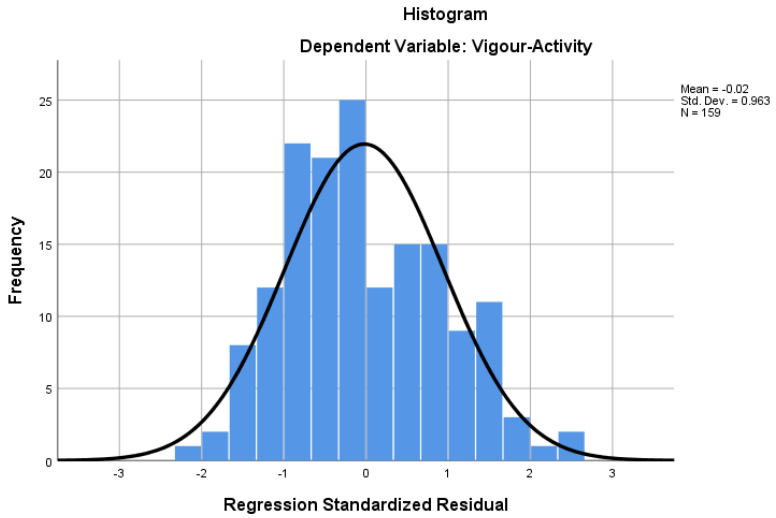
Coefficient Correlations[a]

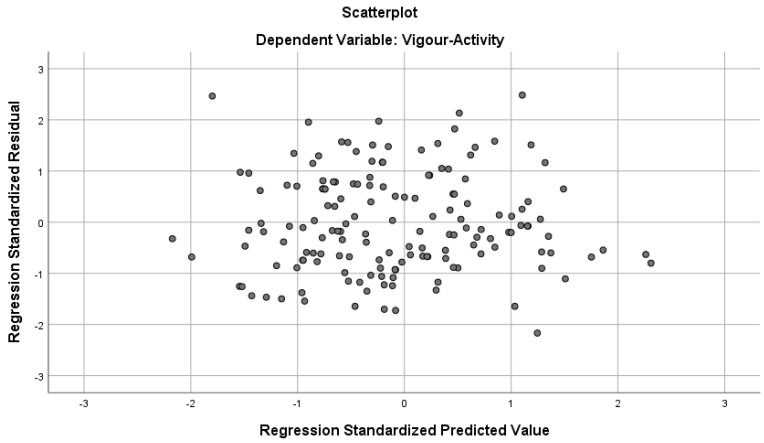
Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.020	1.890E-5	-.001	-3.659E-6	-.001	-.001	.000
		BMI	1.890E-5	.018	-.002	9.878E-7	-.002	-.002	-2.912E-5
		MCQ	-.001	-.002	.046	-3.344E-6	-.001	6.126E-5	.001
		IPAQ	-3.659E-6	9.878E-7	-3.344E-6	5.900E-8	3.296E-7	2.023E-6	1.036E-7
		Haemoglobin	-.001	-.002	-.001	3.296E-7	.007	.001	.000
		Age	-.001	-.002	6.126E-5	2.023E-6	.001	.004	.000
		Ferritin	.000	-2.912E-5	.001	1.036E-7	.000	.000	.000

a. Dependent Variable: Vigour-Activity

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.129	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.859	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.288	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.468	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.200	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Vigour-Activity





Fatigue-Inertia

Correlations

		Fatigue-Inertia	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Fatigue-Inertia	1.000	.103	.005	-.064	-.129	.082	.052	.040
	Haemoglobin	.103	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	.005	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.064	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.129	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.082	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	.052	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	.040	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Fatigue-Inertia	.	.060	.472	.166	.025	.112	.250	.273
	Haemoglobin	.060	.	.000	.126	.016	.431	.425	.211
	Ferritin	.472	.000	.	.001	.060	.132	.014	.371
	Age	.166	.126	.001	.	.000	.012	.323	.226
	BMI	.025	.016	.060	.000	.	.152	.263	.376
	IPAQ	.112	.431	.132	.012	.152	.	.167	.071
	MCQ	.250	.425	.014	.323	.263	.167	.	.283
	Iron	.273	.211	.371	.226	.376	.071	.283	.
N	Fatigue-Inertia	231	231	231	231	231	221	168	226
	Haemoglobin	231	231	231	231	231	221	168	226
	Ferritin	231	231	231	231	231	221	168	226
	Age	231	231	231	231	231	221	168	226
	BMI	231	231	231	231	231	221	168	226
	IPAQ	221	221	221	221	221	221	161	217

	MCQ	168	168	168	168	168	161	168	165
	Iron	226	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Fatigue-Inertia

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.201[a]	.040	-.003	5.145	.040	.921	7	153	.492	2.126

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Fatigue-Inertia

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	170.604	7	24.372	.921	.492[b]
	Residual	4050.167	153	26.472		
	Total	4220.772	160			

a. Dependent Variable: Fatigue-Inertia

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.564	8.579		-.182	.856					
	Haemoglobin	.095	.066	.120	1.423	.157	.103	.114	.113	.878	1.138
	Ferritin	.000	.014	.002	.026	.980	.005	.002	.002	.848	1.179
	Age	-.004	.051	-.006	-.073	.942	-.064	-.006	-.006	.851	1.175
	BMI	-.189	.110	-.143	-1.707	.090	-.129	-.137	-.135	.896	1.116
	IPAQ	.000	.000	.066	.818	.415	.082	.066	.065	.959	1.043
	MCQ	.112	.176	.051	.637	.525	.052	.051	.050	.958	1.043
	Iron	.041	.116	.028	.355	.723	.040	.029	.028	.979	1.022

a. Dependent Variable: Fatigue-Inertia

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.013	1.271E-5	-.001	-2.461E-6	-.001	.000	6.774E-5
		BMI	1.271E-5	.012	-.001	6.644E-7	-.001	-.002	-1.958E-5
		MCQ	-.001	-.001	.031	-2.249E-6	-.001	4.120E-5	.000
		IPAQ	-2.461E-6	6.644E-7	-2.249E-6	3.968E-8	2.217E-7	1.360E-6	6.965E-8
		Haemoglobin	-.001	-.001	-.001	2.217E-7	.004	.001	.000
		Age	.000	-.002	4.120E-5	1.360E-6	.001	.003	.000
		Ferritin	6.774E-5	-1.958E-5	.000	6.965E-8	.000	.000	.000

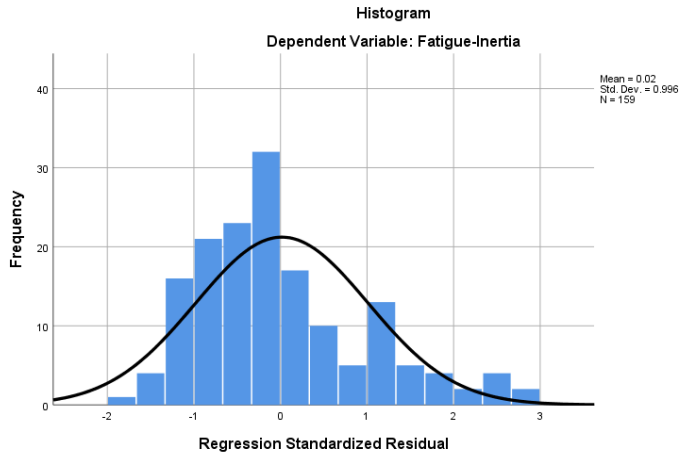
a. Dependent Variable: Fatigue-Inertia

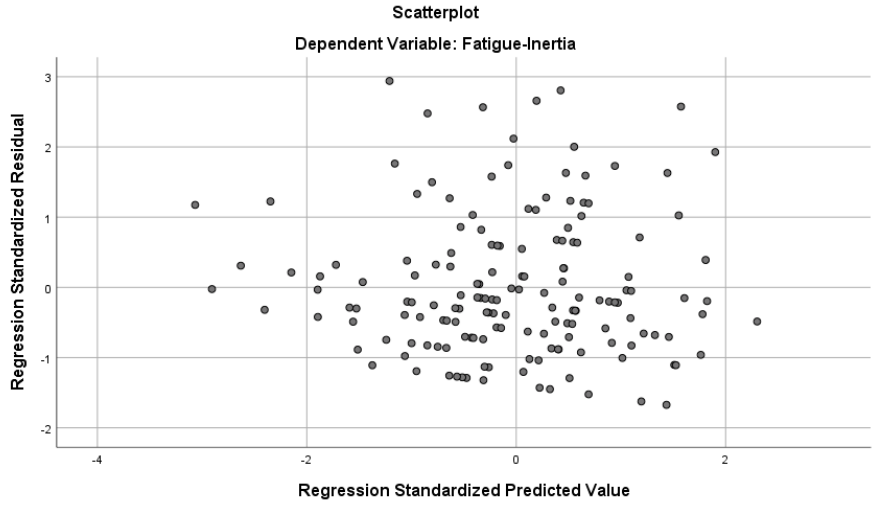
Collinearity Diagnostics[a]

				Variance Proportions							
Model	Dimension	Eigenvalue	Condition Index	(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00

	2	.407	4.129	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.859	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.288	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.468	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.200	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Fatigue-Inertia





Confusion-Bewilderment

Correlations

		Confusion-Bewilderment	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Confusion-Bewilderment	1.000	.057	-.054	-.101	-.121	.018	.062	.080
	Haemoglobin	.057	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	-.054	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.101	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.121	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.018	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	.062	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	.080	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	Confusion-Bewilderment	.	.195	.209	.063	.033	.397	.212	.115
	Haemoglobin	.195	.	.000	.126	.016	.431	.425	.211
	Ferritin	.209	.000	.	.001	.060	.132	.014	.371
	Age	.063	.126	.001	.	.000	.012	.323	.226
	BMI	.033	.016	.060	.000	.	.152	.263	.376
	IPAQ	.397	.431	.132	.012	.152	.	.167	.071
	MCQ	.212	.425	.014	.323	.263	.167	.	.283
	Iron	.115	.211	.371	.226	.376	.071	.283	.
N	Confusion-Bewilderment	231	231	231	231	231	221	168	226
	Haemoglobin	231	231	231	231	231	221	168	226
	Ferritin	231	231	231	231	231	221	168	226
	Age	231	231	231	231	231	221	168	226
	BMI	231	231	231	231	231	221	168	226

	IPAQ	221	221	221	221	221	221	161	217
	MCQ	168	168	168	168	168	161	168	165
	Iron	226	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Confusion-Bewilderment

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.190[a]	.036	-.008	5.107	.036	.818	7	153	.574	1.848

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: Confusion-Bewilderment

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	149.308	7	21.330	.818	.574[b]
	Residual	3990.275	153	26.080		
	Total	4139.582	160			

a. Dependent Variable: Confusion-Bewilderment

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	5.116	8.515		.601	.549					
	Haemoglobin	.058	.066	.074	.878	.382	.057	.071	.070	.878	1.138
	Ferritin	-.006	.014	-.040	-.463	.644	-.054	-.037	-.037	.848	1.179
	Age	-.035	.051	-.060	-.698	.486	-.101	-.056	-.055	.851	1.175
	BMI	-.152	.110	-.116	-1.388	.167	-.121	-.112	-.110	.896	1.116
	IPAQ	-3.381E-5	.000	-.014	-.171	.864	.018	-.014	-.014	.959	1.043
	MCQ	.119	.175	.055	.683	.495	.062	.055	.054	.958	1.043
	Iron	.114	.115	.080	.992	.323	.080	.080	.079	.979	1.022

a. Dependent Variable: Confusion-Bewilderment

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.013	1.252E-5	-.001	-2.425E-6	-.001	.000	6.673E-5
		BMI	1.252E-5	.012	-.001	6.546E-7	-.001	-.002	-1.929E-5
		MCQ	-.001	-.001	.031	-2.216E-6	-.001	4.059E-5	.000

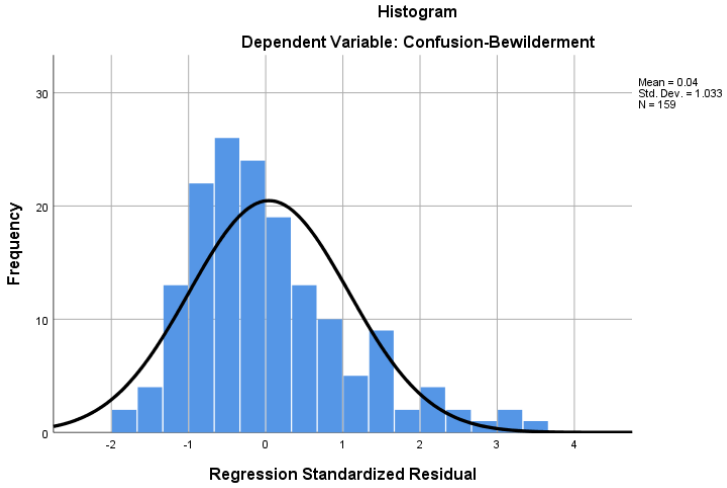
	IPAQ	-2.425E-6	6.546E-7	-2.216E-6	3.910E-8	2.184E-7	1.340E-6	6.862E-8
	Haemoglobin	-.001	-.001	-.001	2.184E-7	.004	.001	.000
	Age	.000	-.002	4.059E-5	1.340E-6	.001	.003	.000
	Ferritin	6.673E-5	-1.929E-5	.000	6.862E-8	.000	.000	.000

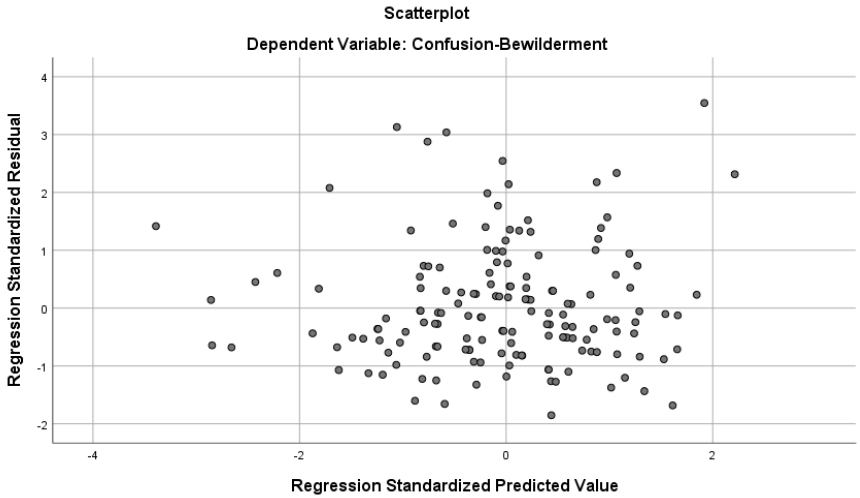
a. Dependent Variable: Confusion-Bewilderment

Collinearity Diagnostics[a]

				Variance Proportions							
Model	Dimension	Eigenvalue	Condition Index	(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.129	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.910	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.859	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.016	.00	.00	.04	.26	.01	.03	.00	.73
	6	.055	11.288	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.468	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.200	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: Confusion-Bewilderment


Histogram
Dependent Variable: Confusion-Bewilderment
Mean = 0.04
Std. Dev. = 1.033
N = 159
Frequency
Regression Standardized Residual


Scatterplot
Dependent Variable: Confusion-Bewilderment
Regression Standardized Residual
Regression Standardized Predicted Value

Total Mood Disturbance

Correlations

		TMD	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	TMD	1.000	.084	-.008	-.187	-.138	.064	-.111	-.029
	Haemoglobin	.084	1.000	.272	-.076	.141	-.012	.015	.054
	Ferritin	-.008	.272	1.000	.198	.103	-.075	-.169	-.022
	Age	-.187	-.076	.198	1.000	.271	-.151	-.036	.050
	BMI	-.138	.141	.103	.271	1.000	-.069	.049	.021
	IPAQ	.064	-.012	-.075	-.151	-.069	1.000	.077	.100
	MCQ	-.111	.015	-.169	-.036	.049	.077	1.000	.045
	Iron	-.029	.054	-.022	.050	.021	.100	.045	1.000
Sig. (1-tailed)	TMD	.	.111	.455	.003	.023	.182	.085	.338
	Haemoglobin	.111	.	.000	.126	.016	.431	.425	.211
	Ferritin	.455	.000	.	.001	.060	.132	.014	.371
	Age	.003	.126	.001	.	.000	.012	.323	.226
	BMI	.023	.016	.060	.000	.	.152	.263	.376
	IPAQ	.182	.431	.132	.012	.152	.	.167	.071
	MCQ	.085	.425	.014	.323	.263	.167	.	.283
	Iron	.338	.211	.371	.226	.376	.071	.283	.
N	TMD	210	210	210	210	210	202	153	208
	Haemoglobin	210	231	231	231	231	221	168	226
	Ferritin	210	231	231	231	231	221	168	226
	Age	210	231	231	231	231	221	168	226
	BMI	210	231	231	231	231	221	168	226
	IPAQ	202	221	221	221	221	221	161	217

	MCQ	153	168	168	168	168	161	168	165
	Iron	208	226	226	226	226	217	165	226

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin[b]	.	Enter

a. Dependent Variable: TMD

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.257[a]	.066	.021	17.235	.066	1.468	7	145	.183	1.968

a. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

b. Dependent Variable: TMD

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3052.561	7	436.080	1.468	.183[b]
	Residual	43069.981	145	297.034		
	Total	46122.542	152			

a. Dependent Variable: TMD

b. Predictors: (Constant), Iron, BMI, MCQ, IPAQ, Haemoglobin, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.651	29.484		.022	.982					
	Haemoglobin	.251	.228	.094	1.099	.273	.084	.091	.088	.878	1.138
	Ferritin	-.006	.048	-.011	-.125	.901	-.008	-.010	-.010	.848	1.179
	Age	-.297	.176	-.147	-1.688	.094	-.187	-.139	-.135	.851	1.175
	BMI	-.450	.380	-.100	-1.185	.238	-.138	-.098	-.095	.896	1.116
	IPAQ	.000	.001	.047	.572	.568	.064	.047	.046	.959	1.043
	MCQ	-.867	.605	-.117	-1.432	.154	-.111	-.118	-.115	.958	1.043
	Iron	-.120	.398	-.024	-.301	.764	-.029	-.025	-.024	.979	1.022

a. Dependent Variable: TMD

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	IPAQ	Haemoglobin	Age	Ferritin
1	Correlations	Iron	1.000	.001	-.031	-.107	-.069	-.077	.041
		BMI	.001	1.000	-.064	.030	-.156	-.271	-.013
		MCQ	-.031	-.064	1.000	-.064	-.052	.005	.173
		IPAQ	-.107	.030	-.064	1.000	.017	.133	.025
		Haemoglobin	-.069	-.156	-.052	.017	1.000	.178	-.295
		Age	-.077	-.271	.005	.133	.178	1.000	-.209
		Ferritin	.041	-.013	.173	.025	-.295	-.209	1.000
	Covariances	Iron	.159	.000	-.007	-2.907E-5	-.006	-.005	.001
		BMI	.000	.144	-.015	7.847E-6	-.014	-.018	.000
		MCQ	-.007	-.015	.366	-2.656E-5	-.007	.000	.005

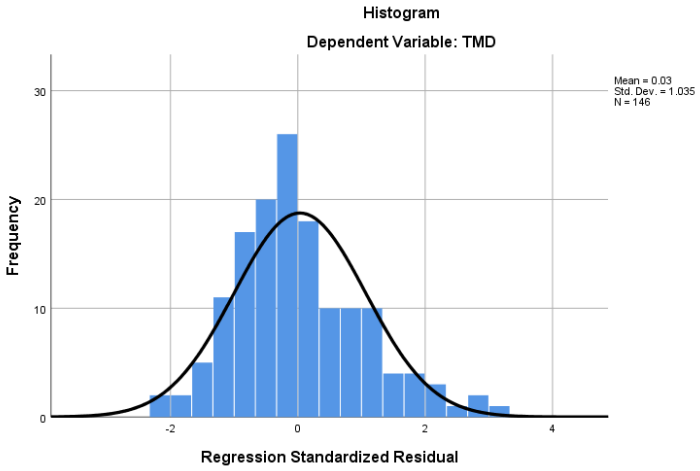
	IPAQ	-2.907E-5	7.847E-6	-2.656E-5	4.687E-7	2.618E-6	1.607E-5	8.227E-7
	Haemoglobin	-.006	-.014	-.007	2.618E-6	.052	.007	-.003
	Age	-.005	-.018	.000	1.607E-5	.007	.031	-.002
	Ferritin	.001	.000	.005	8.227E-7	-.003	-.002	.002

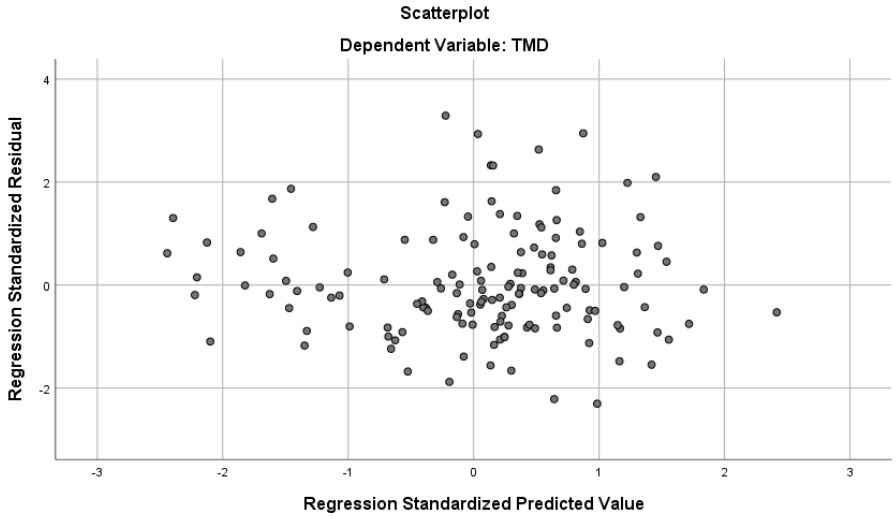
a. Dependent Variable: TMD

Collinearity Diagnostics[a]

				Variance Proportions							
Model	Dimension	Eigenvalue	Condition Index	(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.946	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.407	4.130	.00	.00	.38	.00	.00	.07	.27	.00
	3	.288	4.911	.00	.00	.01	.00	.00	.66	.34	.00
	4	.202	5.860	.00	.00	.49	.03	.01	.16	.36	.05
	5	.085	9.017	.00	.00	.04	.26	.01	.03	.00	.73
	6	.054	11.290	.01	.01	.00	.63	.06	.05	.03	.19
	7	.015	21.472	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	77.214	.97	.97	.07	.05	.00	.00	.00	.00

a. Dependent Variable: TMD


Histogram
Dependent Variable: TMD
Mean = 0.03
Std. Dev. = 1.035
N = 146
Frequency
30
20
10
0
-2
0
2
4
Regression Standardized Residual


Scatterplot
Dependent Variable: TMD
Regression Standardized Residual
4
2
0
-2
-3
-2
-1
0
1
2
3
Regression Standardized Predicted Value

Predictors of subjective mood (PSS and SCI)

PSS

Correlations

		PSS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	PSS	1.000	.095	-.107	-.128	-.004	-.039	.176	-.002
	Haemoglobin	.095	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.107	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.128	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.004	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	-.039	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.176	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	-.002	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	PSS	.	.074	.052	.025	.479	.281	.011	.486
	Haemoglobin	.074	.	.000	.098	.026	.374	.488	.312
	Ferritin	.052	.000	.	.003	.055	.087	.012	.322
	Age	.025	.098	.003	.	.000	.018	.317	.152
	BMI	.479	.026	.055	.000	.	.156	.248	.378
	IPAQ	.281	.374	.087	.018	.156	.	.091	.032
	MCQ	.011	.488	.012	.317	.248	.091	.	.157
	Iron	.486	.312	.322	.152	.378	.032	.157	.
N	PSS	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230

	IPAQ	225	225	225	225	225	225	163	221
	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: PSS

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.256[a]	.066	.023	6.954	.066	1.556	7	155	.153	1.897

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: PSS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	526.684	7	75.241	1.556	.153[b]
	Residual	7495.353	155	48.357		
	Total	8022.037	162			

a. Dependent Variable: PSS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	5.116	11.504		.445	.657					
	Haemoglobin	.116	.088	.108	1.317	.190	.095	.105	.102	.893	1.120
	Ferritin	-.021	.019	-.095	-1.130	.260	-.107	-.090	-.088	.861	1.161
	Age	-.086	.067	-.107	-1.285	.201	-.128	-.103	-.100	.865	1.156
	BMI	.011	.148	.006	.076	.939	-.004	.006	.006	.907	1.103
	IPAQ	.000	.000	-.076	-.956	.340	-.039	-.077	-.074	.950	1.052
	MCQ	.483	.235	.164	2.054	.042	.176	.163	.159	.951	1.051
	Iron	-.009	.155	-.005	-.061	.952	-.002	-.005	-.005	.969	1.032

a. Dependent Variable: PSS

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.024	5.528E-5	-.002	-.001	-5.343E-6	-.001	.000
		BMI	5.528E-5	.022	-.003	-.002	1.325E-6	-.003	-8.062E-5
		MCQ	-.002	-.003	.055	-.001	-5.353E-6	.000	.001

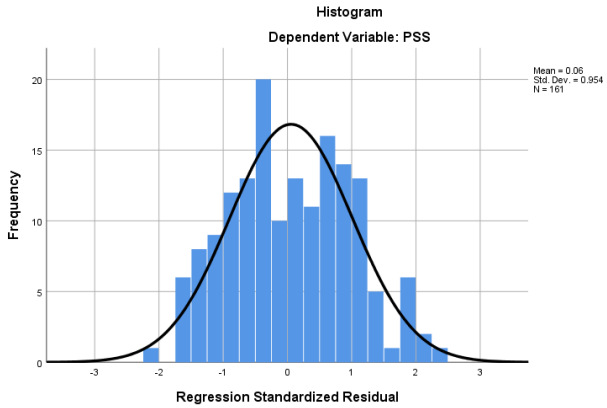
	Haemoglobin	-.001	-.002	-.001	.008	5.048E-7	.001	.000
	IPAQ	-5.343E-6	1.325E-6	-5.353E-6	5.048E-7	7.156E-8	2.256E-6	1.858E-7
	Age	-.001	-.003	.000	.001	2.256E-6	.005	.000
	Ferritin	.000	-8.062E-5	.001	.000	1.858E-7	.000	.000

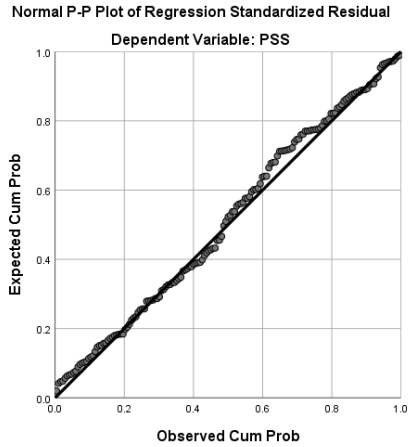
a. Dependent Variable: PSS

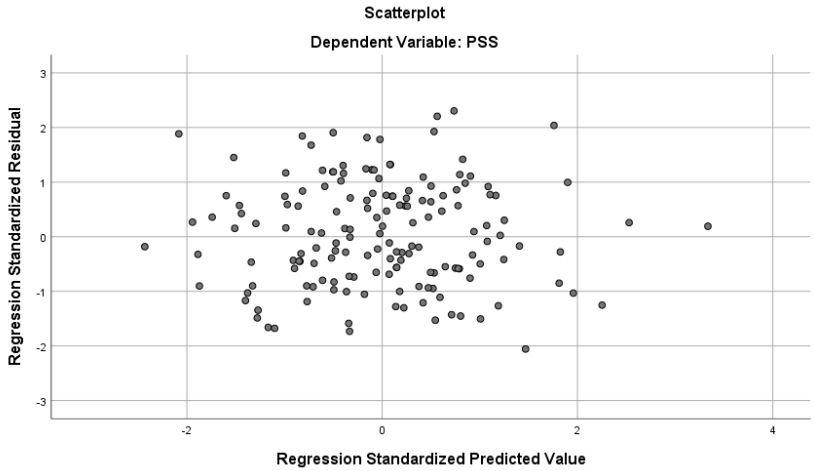
Collinearity Diagnostics[a]

				Variance Proportions							
Model	Dimension	Eigenvalue	Condition Index	(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: PSS


Histogram
Dependent Variable: PSS
Mean = 0.06
Std. Dev. = 0.954
N = 161
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: PSS
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: PSS
Regression Standardized Residual
Regression Standardized Predicted Value

SCI

Correlations

		SCI	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	SCI	1.000	-.021	.062	.041	-.085	-.062	-.279	.089
	Haemoglobin	-.021	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	.062	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	.041	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.085	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	-.062	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	-.279	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.089	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	SCI	.	.373	.174	.267	.098	.178	.000	.088
	Haemoglobin	.373	.	.000	.098	.026	.374	.488	.312
	Ferritin	.174	.000	.	.003	.055	.087	.012	.322
	Age	.267	.098	.003	.	.000	.018	.317	.152
	BMI	.098	.026	.055	.000	.	.156	.248	.378
	IPAQ	.178	.374	.087	.018	.156	.	.091	.032
	MCQ	.000	.488	.012	.317	.248	.091	.	.157
	Iron	.088	.312	.322	.152	.378	.032	.157	.
N	SCI	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221

	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: SCI

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.316[a]	.100	.059	6.812	.100	2.458	7	155	.020	1.940

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: SCI

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	798.353	7	114.050	2.458	.020[b]
	Residual	7191.950	155	46.400		
	Total	7990.303	162			

a. Dependent Variable: SCI

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	27.208	11.268		2.415	.017					
	Haemoglobin	-.020	.086	-.019	-.233	.816	-.021	-.019	-.018	.893	1.120
	Ferritin	.005	.018	.021	.259	.796	.062	.021	.020	.861	1.161
	Age	.026	.066	.032	.391	.696	.041	.031	.030	.865	1.156
	BMI	-.151	.145	-.084	-1.046	.297	-.085	-.084	-.080	.907	1.103
	IPAQ	.000	.000	-.047	-.605	.546	-.062	-.049	-.046	.950	1.052
	MCQ	-.807	.230	-.274	-3.506	.001	-.279	-.271	-.267	.951	1.051
	Iron	.231	.152	.118	1.519	.131	.089	.121	.116	.969	1.032

a. Dependent Variable: SCI

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.023	5.304E-5	-.002	-.001	-5.127E-6	-.001	.000
		BMI	5.304E-5	.021	-.002	-.002	1.272E-6	-.002	-7.736E-5
		MCQ	-.002	-.002	.053	-.001	-5.136E-6	.000	.001

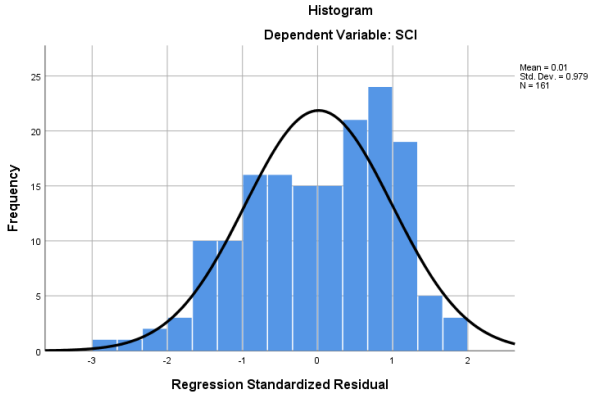
	Haemoglobin	-.001	-.002	-.001	.007	4.844E-7	.001	.000
	IPAQ	-5.127E-6	1.272E-6	-5.136E-6	4.844E-7	6.866E-8	2.165E-6	1.783E-7
	Age	-.001	-.002	.000	.001	2.165E-6	.004	.000
	Ferritin	.000	-7.736E-5	.001	.000	1.783E-7	.000	.000

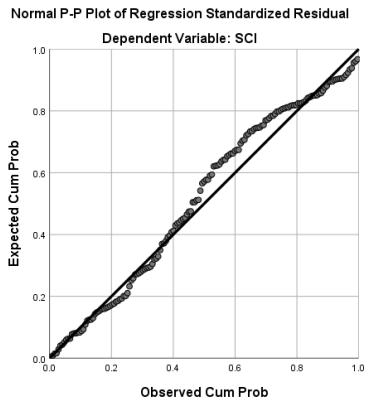
a. Dependent Variable: SCI

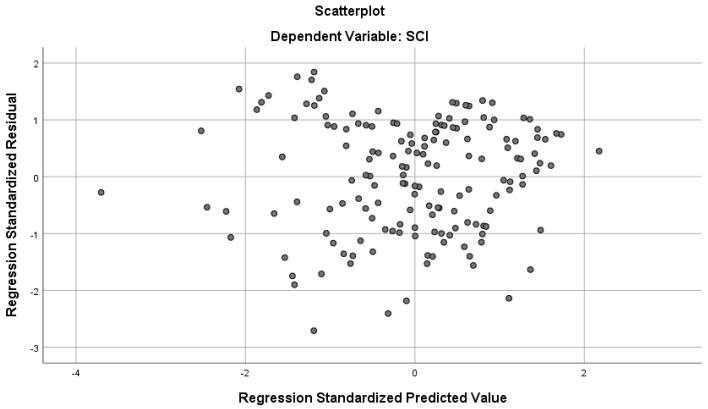
Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: SCI


Histogram
Dependent Variable: SCI
Mean = 0.01
Std. Dev. = 0.979
N = 161
Frequency
Regression Standardized Residual


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: SCI
Expected Cum Prob
Observed Cum Prob


Scatterplot
Dependent Variable: SCI
Regression Standardized Residual
Regression Standardized Predicted Value

Predictors of subjective workload (NASA-TLX)

Correlations

		Workload_2	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Workload_2	1.000	.104	-.005	-.038	.022	.062	-.056	.075	.074
	Haemoglobin	.104	1.000	.258	-.038	-.085	.127	-.021	-.002	.032
	Ferritin	-.005	.258	1.000	.121	.181	.104	-.091	-.173	-.031
	YIE	-.038	-.038	.121	1.000	.115	.046	-.055	-.021	.125
	Age	.022	-.085	.181	.115	1.000	.255	-.140	-.037	.068
	BMI	.062	.127	.104	.046	.255	1.000	-.068	.053	.021
	IPAQ	-.056	-.021	-.091	-.055	-.140	-.068	1.000	.105	.125
	MCQ	.075	-.002	-.173	-.021	-.037	.053	.105	1.000	.078
	Iron	.074	.032	-.031	.125	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Workload_2	.	.057	.468	.283	.371	.173	.203	.169	.134
	Haemoglobin	.057	.	.000	.284	.098	.026	.374	.488	.312
	Ferritin	.468	.000	.	.033	.003	.055	.087	.012	.322
	YIE	.283	.284	.033	.	.040	.244	.207	.391	.030
	Age	.371	.098	.003	.040	.	.000	.018	.317	.152
	BMI	.173	.026	.055	.244	.000	.	.156	.248	.378
	IPAQ	.203	.374	.087	.207	.018	.156	.	.091	.032
	MCQ	.169	.488	.012	.391	.317	.248	.091	.	.157
	Iron	.134	.312	.322	.030	.152	.378	.032	.157	.
N	Workload_2	230	230	230	229	230	230	220	165	225
	Haemoglobin	230	235	235	234	235	235	225	170	230
	Ferritin	230	235	235	234	235	235	225	170	230
	YIE	229	234	234	234	234	234	224	169	229

	Age	230	235	235	234	235	235	225	170	230
	BMI	230	235	235	234	235	235	225	170	230
	IPAQ	220	225	225	224	225	225	225	163	221
	MCQ	165	170	170	169	170	170	163	170	167
	Iron	225	230	230	229	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Workload_2

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.174[a]	.030	-.020	12.45792	.030	.603	8	154	.774	2.132

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

b. Dependent Variable: Workload_2

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	748.977	8	93.622	.603	.774[b]
	Residual	23900.768	154	155.200		
	Total	24649.745	162			

a. Dependent Variable: Workload_2

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, YIE, IPAQ, Age, Ferritin

Coefficients[a]

		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	28.781	21.893		1.315	.191					
	Haemoglobin	.191	.158	.102	1.207	.229	.104	.097	.096	.889	1.125
	Ferritin	-.010	.034	-.025	-.292	.771	-.005	-.024	-.023	.849	1.178
	YIE	-.236	.406	-.047	-.582	.562	-.038	-.047	-.046	.955	1.048
	Age	.025	.121	.018	.207	.836	.022	.017	.016	.862	1.161
	BMI	.126	.264	.040	.476	.635	.062	.038	.038	.906	1.103
	IPAQ	.000	.000	-.071	-.871	.385	-.056	-.070	-.069	.948	1.055
	MCQ	.362	.421	.070	.859	.391	.075	.069	.068	.951	1.051
	Iron	.267	.280	.077	.952	.343	.074	.076	.076	.952	1.050

a. Dependent Variable: Workload_2

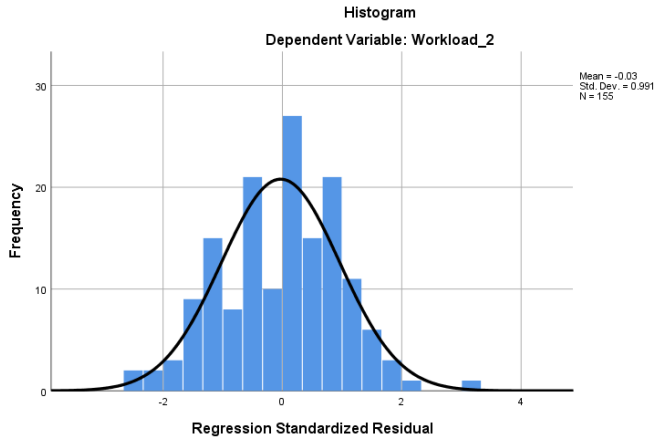
Coefficient Correlations[a]

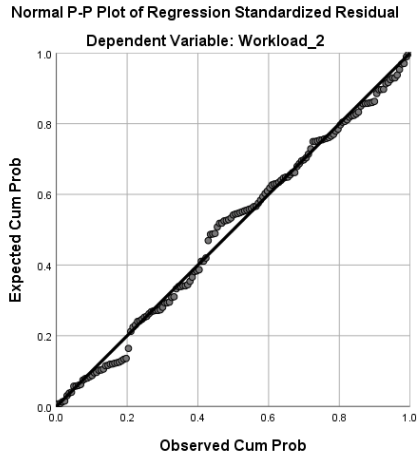
Model			Iron	BMI	MCQ	Haemoglobin	YIE	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.005	-.061	-.061	-.132	-.134	-.086	.053
		BMI	.005	1.000	-.073	-.141	-.019	.033	-.252	-.027
		MCQ	-.061	-.073	1.000	-.031	.004	-.085	.012	.167
		Haemoglobin	-.061	-.141	-.031	1.000	.069	.025	.168	-.281
		YIE	-.132	-.019	.004	.069	1.000	.051	-.062	-.117
		IPAQ	-.134	.033	-.085	.025	.051	1.000	.122	.031
		Age	-.086	-.252	.012	.168	-.062	.122	1.000	-.180
		Ferritin	.053	-.027	.167	-.281	-.117	.031	-.180	1.000
	Covariances	Iron	.079	.000	-.007	-.003	-.015	-1.805E-5	-.003	.000
		BMI	.000	.070	-.008	-.006	-.002	4.133E-6	-.008	.000
		MCQ	-.007	-.008	.177	-.002	.001	-1.714E-5	.001	.002
		Haemoglobin	-.003	-.006	-.002	.025	.004	1.888E-6	.003	-.001
		YIE	-.015	-.002	.001	.004	.165	9.882E-6	-.003	-.002
		IPAQ	-1.805E-5	4.133E-6	-1.714E-5	1.888E-6	9.882E-6	2.302E-7	7.059E-6	5.005E-7
		Age	-.003	-.008	.001	.003	-.003	7.059E-6	.015	-.001
		Ferritin	.000	.000	.002	-.001	-.002	5.005E-7	-.001	.001

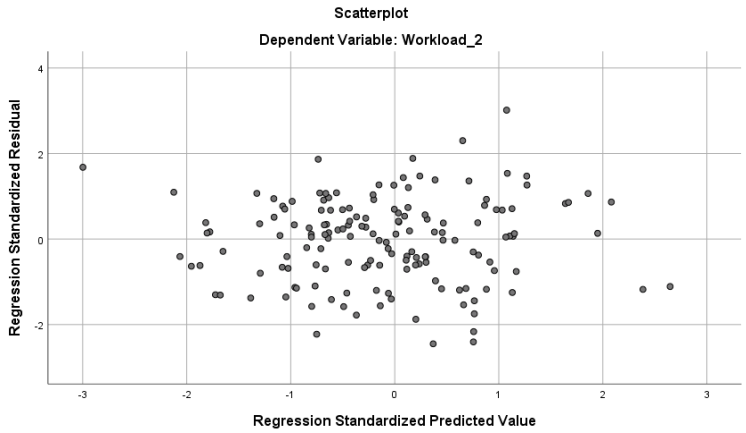
a. Dependent Variable: Workload_2

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	YIE	Age	BMI	IPAQ	MCQ
1	1	7.907	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.350	.00	.00	.36	.00	.00	.00	.08	.25
	3	.282	5.294	.00	.00	.00	.00	.00	.00	.66	.37
	4	.211	6.119	.00	.00	.52	.00	.03	.00	.15	.35
	5	.085	9.661	.00	.00	.03	.00	.23	.01	.04	.00
	6	.063	11.247	.00	.00	.00	.03	.67	.02	.03	.01
	7	.021	19.327	.00	.00	.00	.44	.02	.55	.00	.00
	8	.012	25.870	.03	.06	.00	.47	.01	.41	.02	.00
	9	.001	83.980	.96	.94	.07	.05	.04	.00	.01	.00






Scatterplot
Dependent Variable: Workload_2
Regression Standardized Residual
Regression Standardized Predicted Value
4
2
0
-2
-3
-2
-1
0
1
2
3

Predictors of subjective fatigue (PFS and VAS)
Behavioural/Severity

Correlations

		Behavioural/Severity	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Behavioural/Severity	1.000	.072	-.052	-.074	-.001	.052	.176	.026
	Haemoglobin	.072	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.052	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.074	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.001	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.052	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.176	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.026	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Behavioural/Severity	.	.137	.216	.128	.496	.218	.011	.348
	Haemoglobin	.137	.	.000	.098	.026	.374	.488	.312
	Ferritin	.216	.000	.	.003	.055	.087	.012	.322
	Age	.128	.098	.003	.	.000	.018	.317	.152
	BMI	.496	.026	.055	.000	.	.156	.248	.378
	IPAQ	.218	.374	.087	.018	.156	.	.091	.032
	MCQ	.011	.488	.012	.317	.248	.091	.	.157
	Iron	.348	.312	.322	.152	.378	.032	.157	.
N	Behavioural/Severity	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230

	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221
	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Behavioural/Severity

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.204[a]	.042	-.002	1.92666	.042	.959	7	155	.463	2.011

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Behavioural/Severity

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	24.928	7	3.561	.959	.463[b]
	Residual	575.363	155	3.712		
	Total	600.291	162			

a. Dependent Variable: Behavioural/Severity

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.230	3.187		.072	.943					
	Haemoglobin	.022	.024	.076	.909	.365	.072	.073	.071	.893	1.120
	Ferritin	-.002	.005	-.030	-.357	.722	-.052	-.029	-.028	.861	1.161
	Age	-.012	.019	-.053	-.631	.529	-.074	-.051	-.050	.865	1.156
	BMI	.000	.041	-.001	-.008	.993	-.001	-.001	-.001	.907	1.103
	IPAQ	2.297E-5	.000	.025	.310	.757	.052	.025	.024	.950	1.052
	MCQ	.133	.065	.165	2.050	.042	.176	.162	.161	.951	1.051
	Iron	.005	.043	.010	.126	.900	.026	.010	.010	.969	1.032

a. Dependent Variable: Behavioural/Severity

Coefficient Correlations[a]

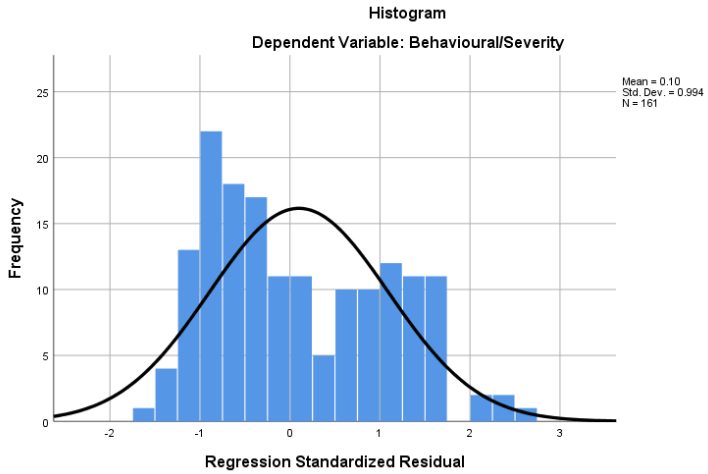
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.002	4.243E-6	.000	-5.520E-5	-4.101E-7	-7.656E-5	8.430E-6
		BMI	4.243E-6	.002	.000	.000	1.017E-7	.000	-6.189E-6
		MCQ	.000	.000	.004	-5.034E-5	-4.109E-7	1.525E-5	5.668E-5
		Haemoglobin	-5.520E-5	.000	-5.034E-5	.001	3.875E-8	7.903E-5	-3.478E-5
		IPAQ	-4.101E-7	1.017E-7	-4.109E-7	3.875E-8	5.493E-9	1.732E-7	1.427E-8
		Age	-7.656E-5	.000	1.525E-5	7.903E-5	1.732E-7	.000	-1.817E-5
		Ferritin	8.430E-6	-6.189E-6	5.668E-5	-3.478E-5	1.427E-8	-1.817E-5	2.666E-5

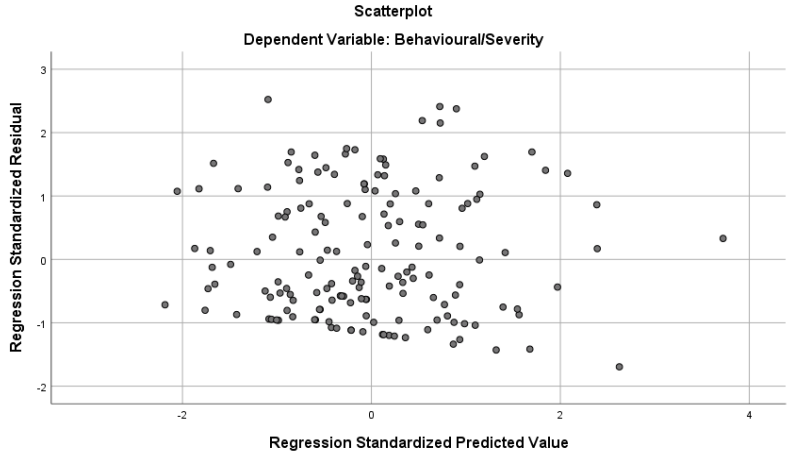
a. Dependent Variable: Behavioural/Severity

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Behavioural/Severity





Affective Meaning

Correlations

		Affective Meaning	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Affective Meaning	1.000	.081	-.063	-.079	-.056	.031	.188	.088
	Haemoglobin	.081	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.063	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.079	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.056	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.031	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.188	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.088	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Affective Meaning	.	.107	.170	.113	.196	.324	.007	.092
	Haemoglobin	.107	.	.000	.098	.026	.374	.488	.312
	Ferritin	.170	.000	.	.003	.055	.087	.012	.322
	Age	.113	.098	.003	.	.000	.018	.317	.152
	BMI	.196	.026	.055	.000	.	.156	.248	.378
	IPAQ	.324	.374	.087	.018	.156	.	.091	.032
	MCQ	.007	.488	.012	.317	.248	.091	.	.157
	Iron	.092	.312	.322	.152	.378	.032	.157	.
N	Affective Meaning	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221

	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Affective Meaning

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.239[a]	.057	.015	2.07254	.057	1.347	7	155	.232	2.154

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Affective Meaning

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	40.494	7	5.785	1.347	.232[b]
	Residual	665.789	155	4.295		
	Total	706.283	162			

a. Dependent Variable: Affective Meaning

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	1.211	3.428		.353	.724					
	Haemoglobin	.030	.026	.093	1.127	.262	.081	.090	.088	.893	1.120
	Ferritin	-.003	.006	-.039	-.464	.643	-.063	-.037	-.036	.861	1.161
	Age	-.012	.020	-.048	-.574	.567	-.079	-.046	-.045	.865	1.156
	BMI	-.034	.044	-.063	-.774	.440	-.056	-.062	-.060	.907	1.103
	IPAQ	-1.008E-5	.000	-.010	-.126	.900	.031	-.010	-.010	.950	1.052
	MCQ	.156	.070	.179	2.233	.027	.188	.177	.174	.951	1.051
	Iron	.044	.046	.076	.955	.341	.088	.076	.074	.969	1.032

a. Dependent Variable: Affective Meaning

Coefficient Correlations[a]

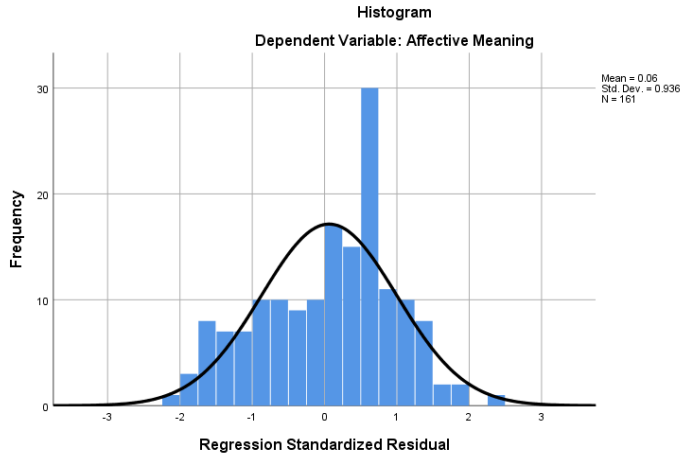
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.002	4.910E-6	.000	-6.387E-5	-4.746E-7	-8.859E-5	9.755E-6
		BMI	4.910E-6	.002	.000	.000	1.177E-7	.000	-7.161E-6
		MCQ	.000	.000	.005	-5.825E-5	-4.755E-7	1.765E-5	6.558E-5
		Haemoglobin	-6.387E-5	.000	-5.825E-5	.001	4.484E-8	9.145E-5	-4.024E-5
		IPAQ	-4.746E-7	1.177E-7	-4.755E-7	4.484E-8	6.356E-9	2.004E-7	1.651E-8
		Age	-8.859E-5	.000	1.765E-5	9.145E-5	2.004E-7	.000	-2.102E-5
		Ferritin	9.755E-6	-7.161E-6	6.558E-5	-4.024E-5	1.651E-8	-2.102E-5	3.085E-5

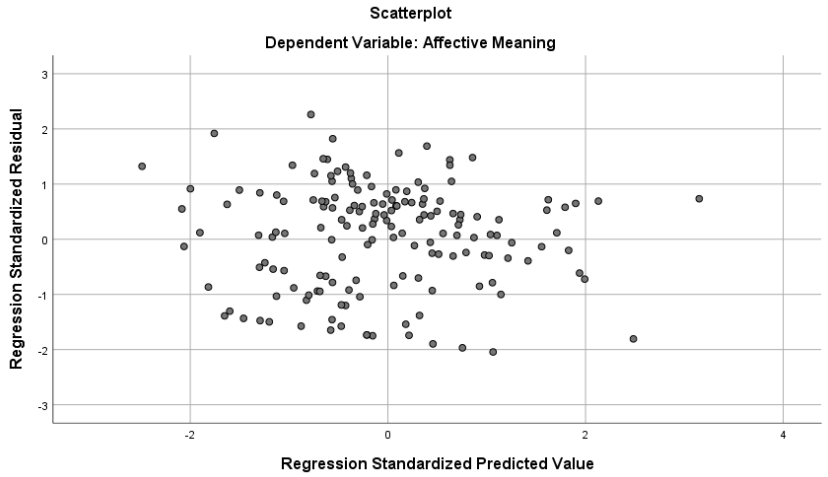
a. Dependent Variable: Affective Meaning

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Affective Meaning





Sensory

Correlations

		Sensory	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Sensory	1.000	.107	-.061	-.154	-.093	.012	.132	-.034
	Haemoglobin	.107	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.061	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.154	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.093	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.012	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.132	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	-.034	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Sensory	.	.052	.177	.009	.077	.432	.043	.302
	Haemoglobin	.052	.	.000	.098	.026	.374	.488	.312
	Ferritin	.177	.000	.	.003	.055	.087	.012	.322
	Age	.009	.098	.003	.	.000	.018	.317	.152
	BMI	.077	.026	.055	.000	.	.156	.248	.378
	IPAQ	.432	.374	.087	.018	.156	.	.091	.032
	MCQ	.043	.488	.012	.317	.248	.091	.	.157
	Iron	.302	.312	.322	.152	.378	.032	.157	.
N	Sensory	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221

	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Sensory

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.241[a]	.058	.016	1.93015	.058	1.371	7	155	.221	2.140

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Sensory

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	35.764	7	5.109	1.371	.221[b]
	Residual	577.449	155	3.725		
	Total	613.213	162			

a. Dependent Variable: Sensory

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	2.604	3.193		.816	.416					
	Haemoglobin	.036	.024	.120	1.458	.147	.107	.116	.114	.893	1.120
	Ferritin	-.003	.005	-.044	-.521	.603	-.061	-.042	-.041	.861	1.161
	Age	-.024	.019	-.110	-1.309	.192	-.154	-.105	-.102	.865	1.156
	BMI	-.042	.041	-.083	-1.020	.309	-.093	-.082	-.079	.907	1.103
	IPAQ	-1.831E-5	.000	-.020	-.247	.806	.012	-.020	-.019	.950	1.052
	MCQ	.106	.065	.130	1.625	.106	.132	.129	.127	.951	1.051
	Iron	-.021	.043	-.038	-.482	.631	-.034	-.039	-.038	.969	1.032

a. Dependent Variable: Sensory

Coefficient Correlations[a]

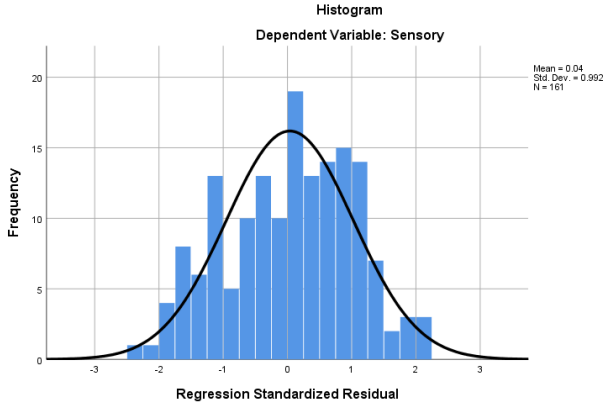
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.002	4.259E-6	.000	-5.540E-5	-4.116E-7	-7.684E-5	8.461E-6
		BMI	4.259E-6	.002	.000	.000	1.021E-7	.000	-6.211E-6
		MCQ	.000	.000	.004	-5.052E-5	-4.124E-7	1.530E-5	5.688E-5
		Haemoglobin	-5.540E-5	.000	-5.052E-5	.001	3.889E-8	7.932E-5	-3.490E-5
		IPAQ	-4.116E-7	1.021E-7	-4.124E-7	3.889E-8	5.513E-9	1.738E-7	1.432E-8
		Age	-7.684E-5	.000	1.530E-5	7.932E-5	1.738E-7	.000	-1.823E-5
		Ferritin	8.461E-6	-6.211E-6	5.688E-5	-3.490E-5	1.432E-8	-1.823E-5	2.676E-5

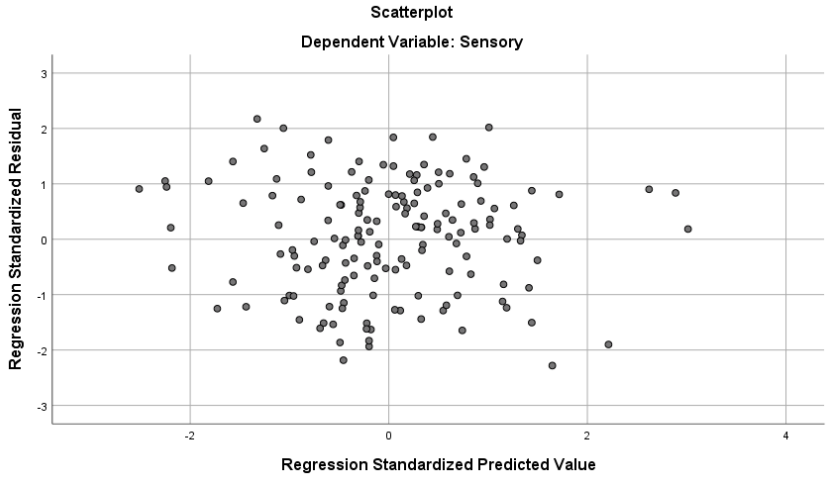
a. Dependent Variable: Sensory

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Sensory





Cognition/Mood

Correlations

		Cognition/Mood	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Cognition/Mood	1.000	.068	-.041	-.022	-.088	.028	.073	.042
	Haemoglobin	.068	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.041	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.022	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.088	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.028	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.073	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.042	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Cognition/Mood	.	.150	.267	.366	.091	.338	.171	.263
	Haemoglobin	.150	.	.000	.098	.026	.374	.488	.312
	Ferritin	.267	.000	.	.003	.055	.087	.012	.322
	Age	.366	.098	.003	.	.000	.018	.317	.152
	BMI	.091	.026	.055	.000	.	.156	.248	.378
	IPAQ	.338	.374	.087	.018	.156	.	.091	.032
	MCQ	.171	.488	.012	.317	.248	.091	.	.157
	Iron	.263	.312	.322	.152	.378	.032	.157	.
N	Cognition/Mood	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221

	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Cognition/Mood

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.153[a]	.023	-.021	1.71951	.023	.532	7	155	.809	1.944

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Cognition/Mood

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.012	7	1.573	.532	.809[b]
	Residual	458.292	155	2.957		
	Total	469.305	162			

a. Dependent Variable: Cognition/Mood

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	2.197	2.845		.772	.441					
	Haemoglobin	.024	.022	.094	1.119	.265	.068	.089	.089	.893	1.120
	Ferritin	-.002	.005	-.044	-.520	.604	-.041	-.042	-.041	.861	1.161
	Age	.004	.017	.022	.258	.797	-.022	.021	.020	.865	1.156
	BMI	-.046	.037	-.104	-1.248	.214	-.088	-.100	-.099	.907	1.103
	IPAQ	8.807E-6	.000	.011	.133	.894	.028	.011	.011	.950	1.052
	MCQ	.049	.058	.069	.842	.401	.073	.067	.067	.951	1.051
	Iron	.015	.038	.031	.390	.697	.042	.031	.031	.969	1.032

a. Dependent Variable: Cognition/Mood

Coefficient Correlations[a]

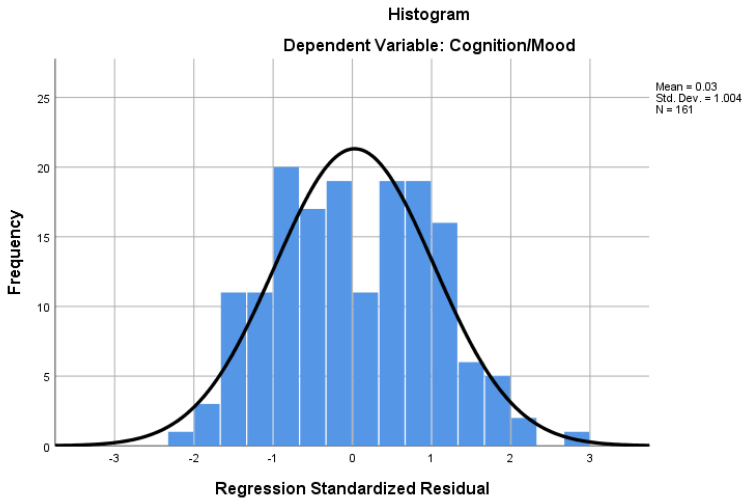
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.001	3.380E-6	.000	-4.397E-5	-3.267E-7	-6.098E-5	6.715E-6
		BMI	3.380E-6	.001	.000	.000	8.102E-8	.000	-4.929E-6
		MCQ	.000	.000	.003	-4.010E-5	-3.273E-7	1.215E-5	4.514E-5
		Haemoglobin	-4.397E-5	.000	-4.010E-5	.000	3.087E-8	6.295E-5	-2.770E-5
		IPAQ	-3.267E-7	8.102E-8	-3.273E-7	3.087E-8	4.375E-9	1.379E-7	1.136E-8
		Age	-6.098E-5	.000	1.215E-5	6.295E-5	1.379E-7	.000	-1.447E-5
		Ferritin	6.715E-6	-4.929E-6	4.514E-5	-2.770E-5	1.136E-8	-1.447E-5	2.124E-5

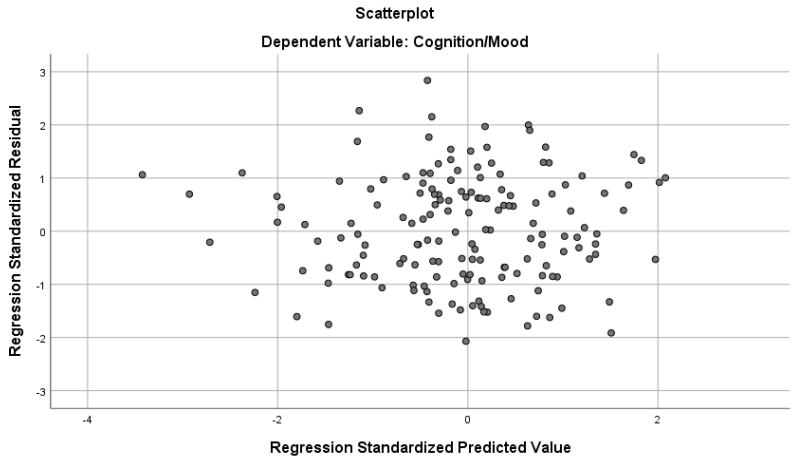
a. Dependent Variable: Cognition/Mood

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Cognition/Mood





Total Fatigue

Correlations

		Total Fatigue	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Total Fatigue	1.000	.091	-.061	-.091	-.064	.035	.160	.035
	Haemoglobin	.091	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.061	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	-.091	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.064	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.035	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	.160	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.035	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Total Fatigue	.	.081	.178	.081	.163	.299	.019	.299
	Haemoglobin	.081	.	.000	.098	.026	.374	.488	.312
	Ferritin	.178	.000	.	.003	.055	.087	.012	.322
	Age	.081	.098	.003	.	.000	.018	.317	.152
	BMI	.163	.026	.055	.000	.	.156	.248	.378
	IPAQ	.299	.374	.087	.018	.156	.	.091	.032
	MCQ	.019	.488	.012	.317	.248	.091	.	.157
	Iron	.299	.312	.322	.152	.378	.032	.157	.
N	Total Fatigue	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221
	MCQ	170	170	170	170	170	163	170	167

Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Total Fatigue

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.216[a]	.047	.004	1.69186	.047	1.085	7	155	.376	2.116

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Total Fatigue

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	21.736	7	3.105	1.085	.376[b]
	Residual	443.670	155	2.862		
	Total	465.406	162			

a. Dependent Variable: Total Fatigue

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	1.530	2.799		.547	.585					
	Haemoglobin	.028	.021	.107	1.285	.201	.091	.103	.101	.893	1.120
	Ferritin	-.002	.005	-.044	-.524	.601	-.061	-.042	-.041	.861	1.161
	Age	-.010	.016	-.053	-.624	.534	-.091	-.050	-.049	.865	1.156
	BMI	-.030	.036	-.068	-.826	.410	-.064	-.066	-.065	.907	1.103
	IPAQ	2.390E-6	.000	.003	.037	.971	.035	.003	.003	.950	1.052
	MCQ	.108	.057	.152	1.886	.061	.160	.150	.148	.951	1.051
	Iron	.011	.038	.023	.288	.774	.035	.023	.023	.969	1.032

a. Dependent Variable: Total Fatigue

Coefficient Correlations[a]

Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.001	3.272E-6	.000	-4.256E-5	-3.163E-7	-5.904E-5	6.501E-6
		BMI	3.272E-6	.001	.000	.000	7.844E-8	.000	-4.772E-6
		MCQ	.000	.000	.003	-3.882E-5	-3.169E-7	1.176E-5	4.370E-5
		Haemoglobin	-4.256E-5	.000	-3.882E-5	.000	2.988E-8	6.094E-5	-2.682E-5
		IPAQ	-3.163E-7	7.844E-8	-3.169E-7	2.988E-8	4.236E-9	1.335E-7	1.100E-8
		Age	-5.904E-5	.000	1.176E-5	6.094E-5	1.335E-7	.000	-1.401E-5
		Ferritin	6.501E-6	-4.772E-6	4.370E-5	-2.682E-5	1.100E-8	-1.401E-5	2.056E-5

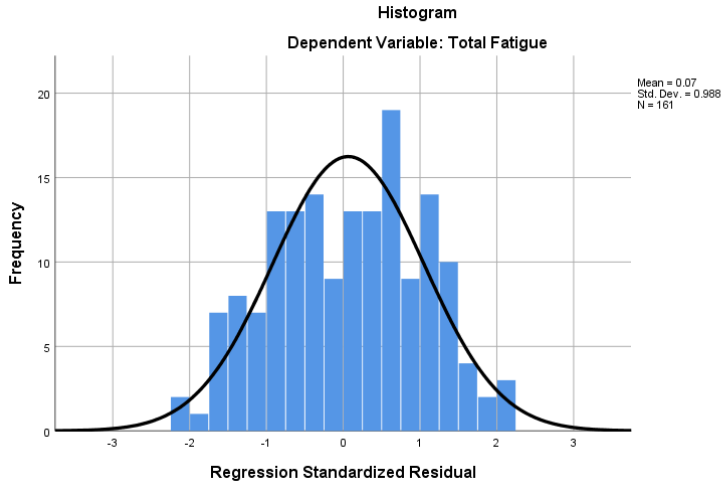
a. Dependent Variable: Total Fatigue

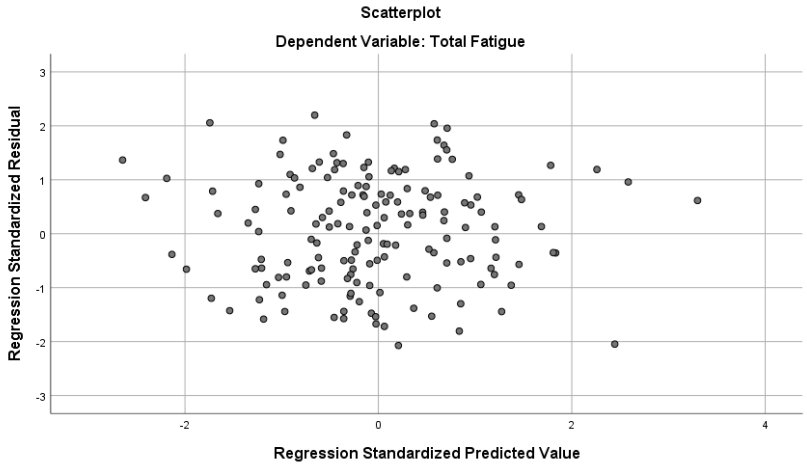
Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions

				(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Total Fatigue





Mental Fatigue (VAS)

Correlations

		Mental_Fatigue	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Mental_Fatigue	1.000	.043	-.073	.013	-.036	-.018	-.001	.042
	Haemoglobin	.043	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	-.073	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	.013	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	-.036	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	-.018	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	-.001	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.042	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Mental_Fatigue	.	.257	.133	.421	.292	.394	.496	.262
	Haemoglobin	.257	.	.000	.098	.026	.374	.488	.312
	Ferritin	.133	.000	.	.003	.055	.087	.012	.322
	Age	.421	.098	.003	.	.000	.018	.317	.152
	BMI	.292	.026	.055	.000	.	.156	.248	.378
	IPAQ	.394	.374	.087	.018	.156	.	.091	.032
	MCQ	.496	.488	.012	.317	.248	.091	.	.157
	Iron	.262	.312	.322	.152	.378	.032	.157	.
N	Mental_Fatigue	235	235	235	235	235	225	170	230
	Haemoglobin	235	235	235	235	235	225	170	230
	Ferritin	235	235	235	235	235	225	170	230
	Age	235	235	235	235	235	225	170	230
	BMI	235	235	235	235	235	225	170	230
	IPAQ	225	225	225	225	225	225	163	221

	MCQ	170	170	170	170	170	163	170	167
	Iron	230	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Mental_Fatigue

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.122[a]	.015	-.030	18.30384	.015	.335	7	155	.937	2.270

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Mental_Fatigue

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	786.797	7	112.400	.335	.937[b]
	Residual	51929.741	155	335.031		
	Total	52716.538	162			

a. Dependent Variable: Mental_Fatigue

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	38.863	30.279		1.283	.201					
	Haemoglobin	.210	.232	.076	.905	.367	.043	.072	.072	.893	1.120
	Ferritin	-.057	.049	-.099	-1.153	.251	-.073	-.092	-.092	.861	1.161
	Age	.089	.177	.043	.501	.617	.013	.040	.040	.865	1.156
	BMI	-.223	.389	-.048	-.573	.567	-.036	-.046	-.046	.907	1.103
	IPAQ	.000	.001	-.026	-.318	.751	-.018	-.026	-.025	.950	1.052
	MCQ	-.106	.619	-.014	-.171	.864	-.001	-.014	-.014	.951	1.051
	Iron	.198	.408	.039	.484	.629	.042	.039	.039	.969	1.032

a. Dependent Variable: Mental_Fatigue

Coefficient Correlations[a]

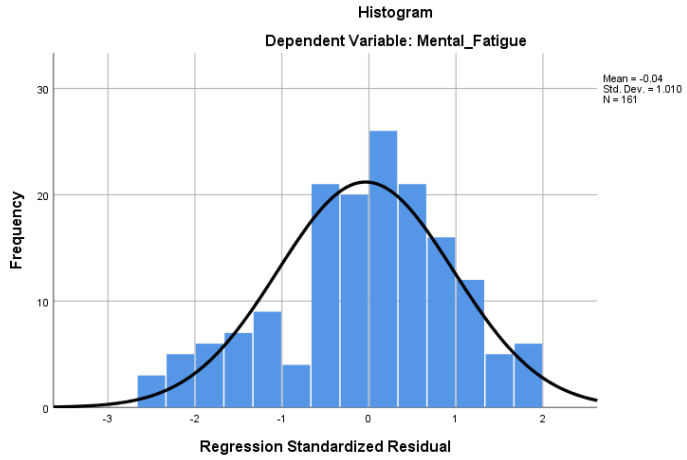
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.167	.000	-.015	-.005	-3.702E-5	-.007	.001
		BMI	.000	.151	-.018	-.013	9.181E-6	-.017	-.001
		MCQ	-.015	-.018	.383	-.005	-3.709E-5	.001	.005
		Haemoglobin	-.005	-.013	-.005	.054	3.498E-6	.007	-.003
		IPAQ	-3.702E-5	9.181E-6	-3.709E-5	3.498E-6	4.958E-7	1.563E-5	1.288E-6
		Age	-.007	-.017	.001	.007	1.563E-5	.031	-.002
		Ferritin	.001	-.001	.005	-.003	1.288E-6	-.002	.002

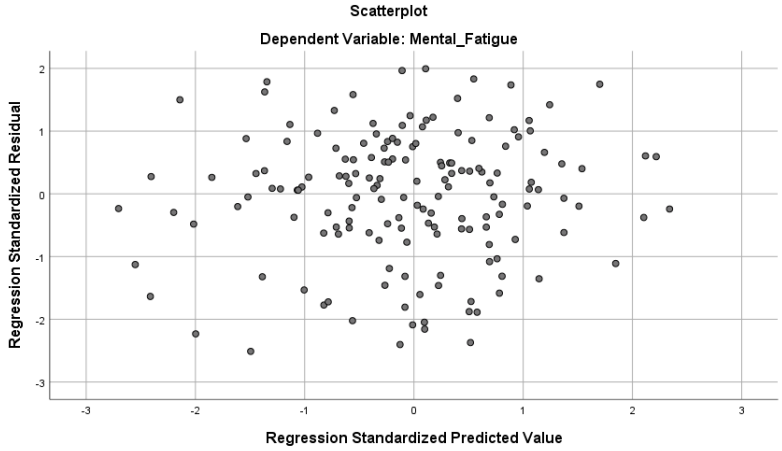
a. Dependent Variable: Mental_Fatigue

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Mental_Fatigue





Alertness

Correlations

		Alertness	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	Alertness	1.000	-.033	.050	.108	.151	.055	-.157	-.037
	Haemoglobin	-.033	1.000	.258	-.085	.127	-.021	-.002	.032
	Ferritin	.050	.258	1.000	.181	.104	-.091	-.173	-.031
	Age	.108	-.085	.181	1.000	.255	-.140	-.037	.068
	BMI	.151	.127	.104	.255	1.000	-.068	.053	.021
	IPAQ	.055	-.021	-.091	-.140	-.068	1.000	.105	.125
	MCQ	-.157	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	-.037	.032	-.031	.068	.021	.125	.078	1.000
Sig. (1-tailed)	Alertness	.	.308	.225	.050	.010	.204	.021	.291
	Haemoglobin	.308	.	.000	.098	.026	.374	.488	.312
	Ferritin	.225	.000	.	.003	.055	.087	.012	.322
	Age	.050	.098	.003	.	.000	.018	.317	.152
	BMI	.010	.026	.055	.000	.	.156	.248	.378
	IPAQ	.204	.374	.087	.018	.156	.	.091	.032
	MCQ	.021	.488	.012	.317	.248	.091	.	.157
	Iron	.291	.312	.322	.152	.378	.032	.157	.
N	Alertness	234	234	234	234	234	224	169	229
	Haemoglobin	234	235	235	235	235	225	170	230
	Ferritin	234	235	235	235	235	225	170	230
	Age	234	235	235	235	235	225	170	230
	BMI	234	235	235	235	235	225	170	230
	IPAQ	224	225	225	225	225	225	163	221

	MCQ	169	170	170	170	170	163	170	167
	Iron	229	230	230	230	230	221	167	230

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: Alertness

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.258[a]	.067	.024	18.18269	.067	1.580	7	155	.145	1.857

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: Alertness

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3656.795	7	522.399	1.580	.145[b]
	Residual	51244.599	155	330.610		
	Total	54901.394	162			

a. Dependent Variable: Alertness

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	37.700	30.079		1.253	.212					
	Haemoglobin	-.129	.231	-.046	-.560	.576	-.033	-.045	-.043	.893	1.120
	Ferritin	.006	.049	.011	.129	.897	.050	.010	.010	.861	1.161
	Age	.154	.176	.073	.877	.382	.108	.070	.068	.865	1.156
	BMI	.728	.386	.154	1.885	.061	.151	.150	.146	.907	1.103
	IPAQ	.001	.001	.099	1.243	.216	.055	.099	.096	.950	1.052
	MCQ	-1.298	.614	-.168	-2.112	.036	-.157	-.167	-.164	.951	1.051
	Iron	-.216	.406	-.042	-.534	.594	-.037	-.043	-.041	.969	1.032

a. Dependent Variable: Alertness

Coefficient Correlations[a]

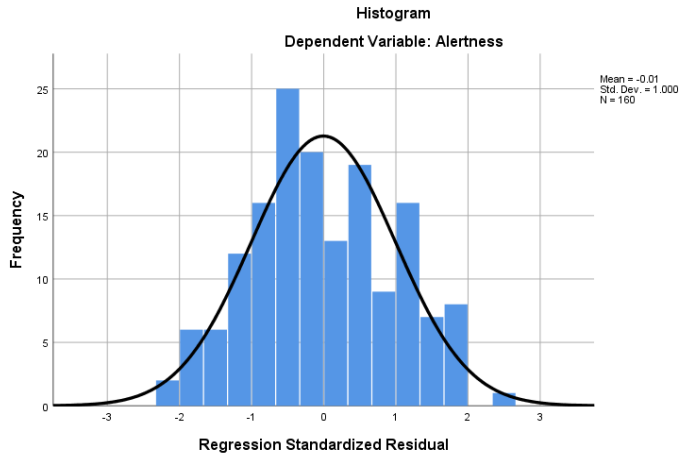
Model			Iron	BMI	MCQ	Haemoglobin	IPAQ	Age	Ferritin
1	Correlations	Iron	1.000	.002	-.061	-.053	-.129	-.096	.038
		BMI	.002	1.000	-.073	-.140	.034	-.254	-.029
		MCQ	-.061	-.073	1.000	-.032	-.085	.013	.169
		Haemoglobin	-.053	-.140	-.032	1.000	.021	.173	-.275
		IPAQ	-.129	.034	-.085	.021	1.000	.125	.037
		Age	-.096	-.254	.013	.173	.125	1.000	-.189
		Ferritin	.038	-.029	.169	-.275	.037	-.189	1.000
	Covariances	Iron	.164	.000	-.015	-.005	-3.653E-5	-.007	.001
		BMI	.000	.149	-.017	-.012	9.060E-6	-.017	-.001
		MCQ	-.015	-.017	.378	-.004	-3.660E-5	.001	.005
		Haemoglobin	-.005	-.012	-.004	.053	3.452E-6	.007	-.003
		IPAQ	-3.653E-5	9.060E-6	-3.660E-5	3.452E-6	4.892E-7	1.542E-5	1.271E-6
		Age	-.007	-.017	.001	.007	1.542E-5	.031	-.002
		Ferritin	.001	-.001	.005	-.003	1.271E-6	-.002	.002

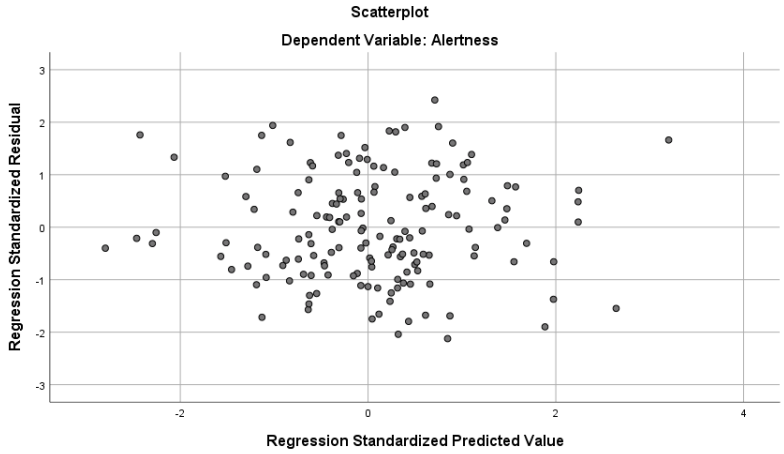
a. Dependent Variable: Alertness

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.939	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.417	4.079	.00	.00	.38	.00	.00	.08	.25	.00
	3	.282	4.961	.00	.00	.00	.00	.00	.65	.38	.00
	4	.203	5.844	.00	.00	.51	.04	.01	.16	.34	.04
	5	.085	9.055	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	11.005	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.307	.03	.03	.00	.02	.93	.01	.00	.03
	8	.001	76.680	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: Alertness





Predictors of subjective wellbeing (SF-12)
Physical functioning

Correlations

		PF_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	PF_NBS	1.000	-.112	-.075	.004	-.112	.115	-.021	.112
	Haemoglobin	-.112	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	-.075	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.004	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.112	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.115	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.021	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.112	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	PF_NBS	.	.043	.128	.473	.044	.043	.393	.045
	Haemoglobin	.043	.	.000	.097	.026	.375	.488	.309
	Ferritin	.128	.000	.	.003	.056	.087	.012	.319
	Age	.473	.097	.003	.	.000	.019	.317	.134
	BMI	.044	.026	.056	.000	.	.159	.248	.365
	IPAQ	.043	.375	.087	.019	.159	.	.091	.034
	MCQ	.393	.488	.012	.317	.248	.091	.	.157
	Iron	.045	.309	.319	.134	.365	.034	.157	.
N	PF_NBS	234	234	234	234	234	224	170	229
	Haemoglobin	234	234	234	234	234	224	170	229
	Ferritin	234	234	234	234	234	224	170	229
	Age	234	234	234	234	234	224	170	229
	BMI	234	234	234	234	234	224	170	229

	IPAQ	224	224	224	224	224	224	163	220
	MCQ	170	170	170	170	170	163	170	167
	Iron	229	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: PF_NBS

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.219[a]	.048	.005	6.21924	.048	1.117	7	155	.355	2.027

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: PF_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	302.337	7	43.191	1.117	.355[b]
	Residual	5995.228	155	38.679		
	Total	6297.566	162			

a. Dependent Variable: PF_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

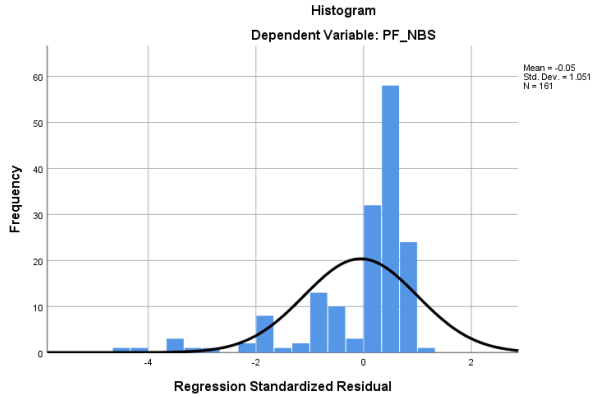
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	65.488	10.268		6.378	.000					
	Haemoglobin	-.083	.079	-.087	-1.050	.295	-.112	-.084	-.082	.893	1.120
	Ferritin	-.008	.017	-.043	-.507	.613	-.075	-.041	-.040	.861	1.162
	Age	.025	.060	.034	.407	.684	.004	.033	.032	.864	1.157
	BMI	-.158	.132	-.099	-1.197	.233	-.112	-.096	-.094	.907	1.102
	IPAQ	.000	.000	.099	1.229	.221	.115	.098	.096	.951	1.051
	MCQ	-.106	.210	-.041	-.505	.614	-.021	-.041	-.040	.951	1.051
	Iron	.182	.139	.104	1.310	.192	.112	.105	.103	.968	1.033

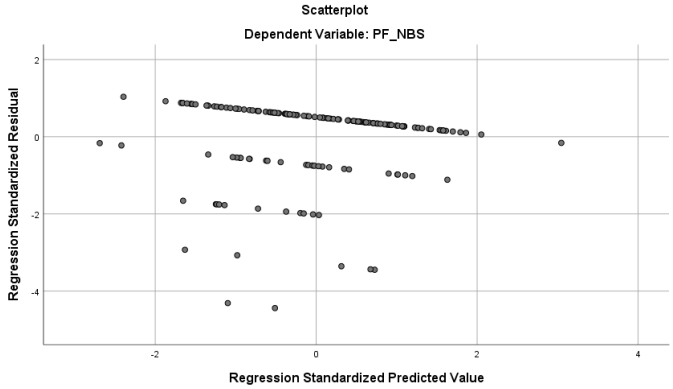
a. Dependent Variable: PF_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: PF_NBS





Role limitations due to physical functioning

Correlations

		RP_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	RP_NBS	1.000	-.023	.070	.004	-.069	.062	-.042	.002
	Haemoglobin	-.023	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.070	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.004	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.069	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.062	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.042	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.002	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	RP_NBS	.	.363	.144	.477	.147	.180	.293	.489
	Haemoglobin	.363	.	.000	.097	.026	.375	.488	.309
	Ferritin	.144	.000	.	.003	.056	.087	.012	.319
	Age	.477	.097	.003	.	.000	.019	.317	.134
	BMI	.147	.026	.056	.000	.	.159	.248	.365
	IPAQ	.180	.375	.087	.019	.159	.	.091	.034
	MCQ	.293	.488	.012	.317	.248	.091	.	.157
	Iron	.489	.309	.319	.134	.365	.034	.157	.
N	RP_NBS	234	234	234	234	234	224	170	229
	Haemoglobin	234	234	234	234	234	224	170	229
	Ferritin	234	234	234	234	234	224	170	229
	Age	234	234	234	234	234	224	170	229
	BMI	234	234	234	234	234	224	170	229
	IPAQ	224	224	224	224	224	224	163	220

	MCQ	170	170	170	170	170	163	170	167
	Iron	229	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: RP_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.131[a]	.017	-.027	7.12640	.017	.384	7	155	.911	1.703

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: RP_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	136.550	7	19.507	.384	.911[b]
	Residual	7871.756	155	50.786		
	Total	8008.306	162			

a. Dependent Variable: RP_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

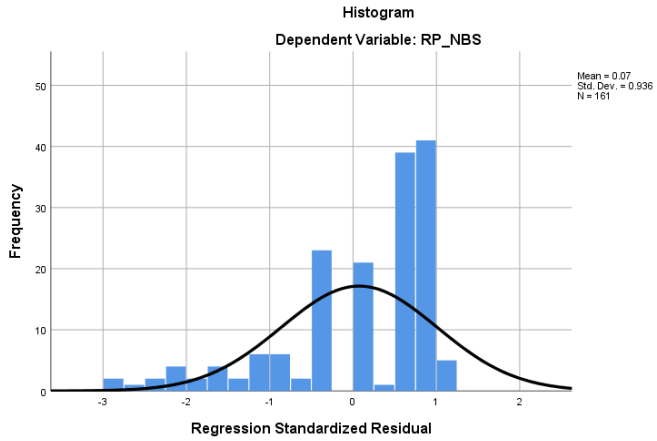
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	58.113	11.766		4.939	.000					
	Haemoglobin	-.036	.090	-.033	-.396	.693	-.023	-.032	-.032	.893	1.120
	Ferritin	.019	.019	.084	.983	.327	.070	.079	.078	.861	1.162
	Age	.010	.069	.012	.138	.890	.004	.011	.011	.864	1.157
	BMI	-.127	.151	-.070	-.841	.402	-.069	-.067	-.067	.907	1.102
	IPAQ	.000	.000	.069	.840	.402	.062	.067	.067	.951	1.051
	MCQ	-.091	.241	-.031	-.376	.707	-.042	-.030	-.030	.951	1.051
	Iron	.001	.159	.000	.003	.997	.002	.000	.000	.968	1.033

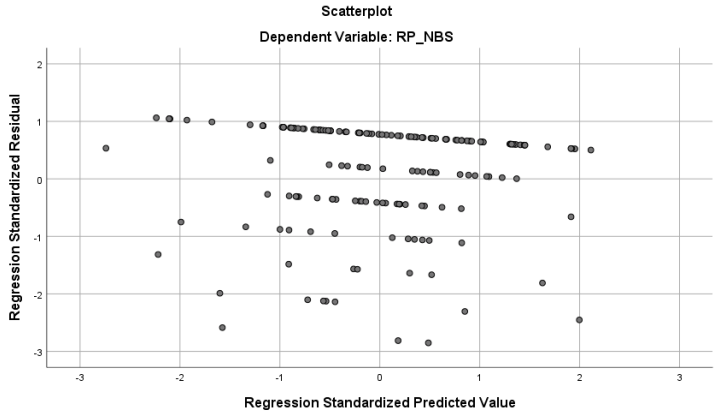
a. Dependent Variable: RP_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: RP_NBS





Bodily Pain

Correlations

		BP_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	BP_NBS	1.000	-.135	-.119	.107	-.051	-.135	-.002	.048
	Haemoglobin	-.135	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	-.119	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.107	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.051	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	-.135	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.002	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.048	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	BP_NBS	.	.020	.034	.052	.217	.022	.491	.236
	Haemoglobin	.020	.	.000	.097	.026	.375	.488	.309
	Ferritin	.034	.000	.	.003	.056	.087	.012	.319
	Age	.052	.097	.003	.	.000	.019	.317	.134
	BMI	.217	.026	.056	.000	.	.159	.248	.365
	IPAQ	.022	.375	.087	.019	.159	.	.091	.034
	MCQ	.491	.488	.012	.317	.248	.091	.	.157
	Iron	.236	.309	.319	.134	.365	.034	.157	.
N	BP_NBS	234	234	234	234	234	224	170	229
	Haemoglobin	234	234	234	234	234	224	170	229
	Ferritin	234	234	234	234	234	224	170	229
	Age	234	234	234	234	234	224	170	229
	BMI	234	234	234	234	234	224	170	229
	IPAQ	224	224	224	224	224	224	163	220

	MCQ	170	170	170	170	170	163	170	167
	Iron	229	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: BP_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.254[a]	.064	.022	6.82418	.064	1.523	7	155	.163	1.972

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: BP_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	496.375	7	70.911	1.523	.163[b]
	Residual	7218.254	155	46.569		
	Total	7714.629	162			

a. Dependent Variable: BP_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

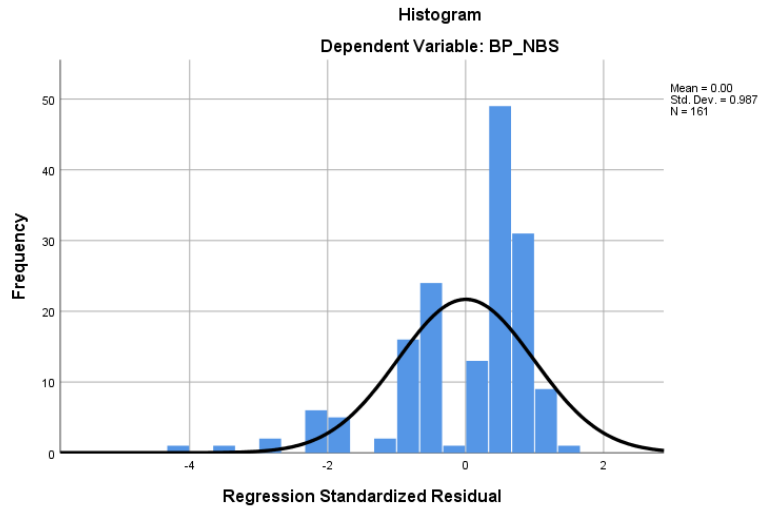
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	67.404	11.267		5.983	.000					
	Haemoglobin	-.095	.086	-.090	-1.094	.275	-.135	-.088	-.085	.893	1.120
	Ferritin	-.027	.018	-.122	-1.456	.147	-.119	-.116	-.113	.861	1.162
	Age	.090	.066	.114	1.361	.175	.107	.109	.106	.864	1.157
	BMI	-.118	.145	-.067	-.819	.414	-.051	-.066	-.064	.907	1.102
	IPAQ	.000	.000	-.143	-1.797	.074	-.135	-.143	-.140	.951	1.051
	MCQ	-.014	.231	-.005	-.062	.951	-.002	-.005	-.005	.951	1.051
	Iron	.112	.153	.058	.736	.463	.048	.059	.057	.968	1.033

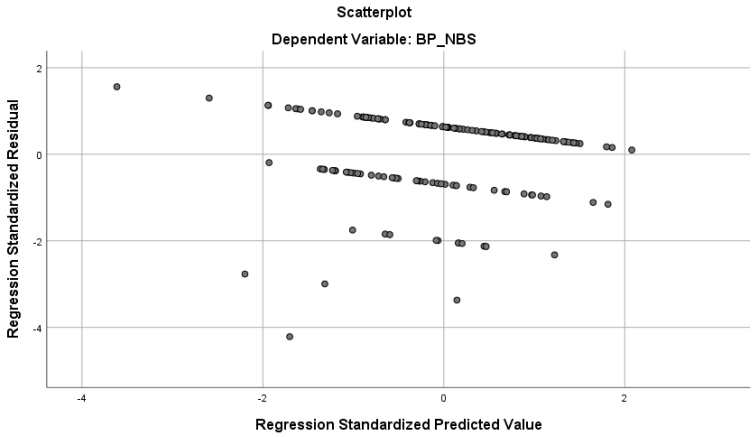
a. Dependent Variable: BP_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: BP_NBS





General Health

Correlations

		GH_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	GH_NBS	1.000	.008	-.031	-.039	-.154	.137	-.104	.092
	Haemoglobin	.008	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	-.031	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	-.039	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.154	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.137	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.104	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.092	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	GH_NBS	.	.450	.320	.275	.010	.020	.090	.082
	Haemoglobin	.450	.	.000	.097	.026	.375	.488	.309
	Ferritin	.320	.000	.	.003	.056	.087	.012	.319
	Age	.275	.097	.003	.	.000	.019	.317	.134
	BMI	.010	.026	.056	.000	.	.159	.248	.365
	IPAQ	.020	.375	.087	.019	.159	.	.091	.034
	MCQ	.090	.488	.012	.317	.248	.091	.	.157
	Iron	.082	.309	.319	.134	.365	.034	.157	.
N	GH_NBS	233	233	233	233	233	223	169	228
	Haemoglobin	233	234	234	234	234	224	170	229
	Ferritin	233	234	234	234	234	224	170	229
	Age	233	234	234	234	234	224	170	229
	BMI	233	234	234	234	234	224	170	229
	IPAQ	223	224	224	224	224	224	163	220

	MCQ	169	170	170	170	170	163	170	167
	Iron	228	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: GH_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.248[a]	.061	.019	8.23403	.061	1.449	7	155	.190	1.971

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: GH_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	687.502	7	98.215	1.449	.190[b]
	Residual	10508.872	155	67.799		
	Total	11196.374	162			

a. Dependent Variable: GH_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

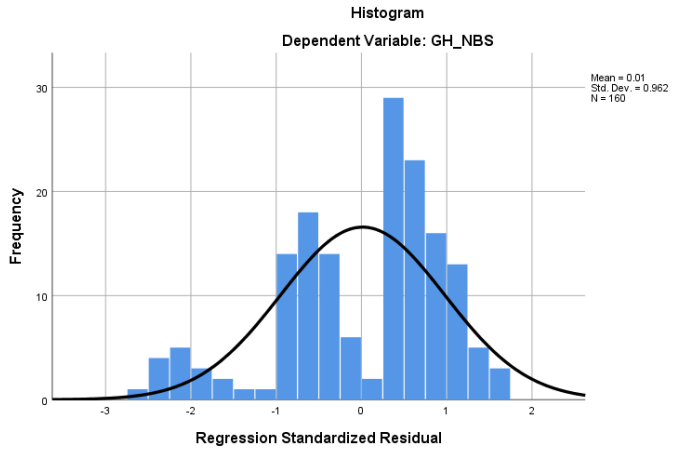
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	51.904	13.595		3.818	.000					
	Haemoglobin	.046	.104	.036	.442	.659	.008	.035	.034	.893	1.120
	Ferritin	-.009	.022	-.034	-.408	.684	-.031	-.033	-.032	.861	1.162
	Age	.013	.080	.014	.166	.868	-.039	.013	.013	.864	1.157
	BMI	-.310	.174	-.145	-1.776	.078	-.154	-.141	-.138	.907	1.102
	IPAQ	.001	.000	.129	1.622	.107	.137	.129	.126	.951	1.051
	MCQ	-.424	.278	-.122	-1.525	.129	-.104	-.122	-.119	.951	1.051
	Iron	.200	.184	.086	1.087	.279	.092	.087	.085	.968	1.033

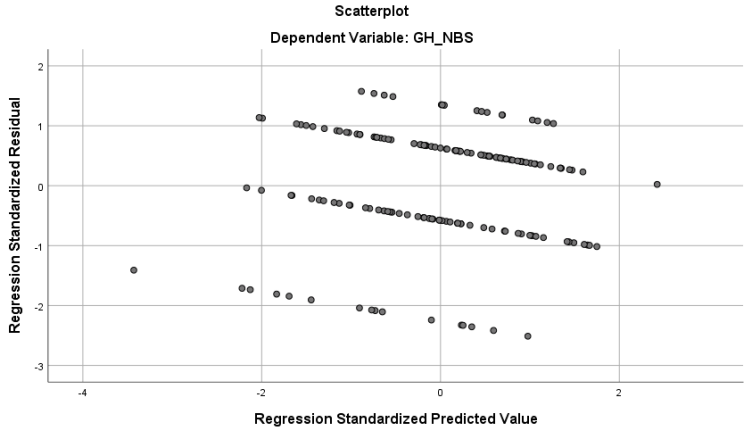
a. Dependent Variable: GH_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: GH_NBS





Vitality

Correlations

		VT_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	VT_NBS	1.000	-.008	.039	-.046	-.192	.173	-.051	.081
	Haemoglobin	-.008	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.039	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	-.046	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.192	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.173	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.051	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.081	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	VT_NBS	.	.452	.279	.244	.002	.005	.253	.112
	Haemoglobin	.452	.	.000	.097	.026	.375	.488	.309
	Ferritin	.279	.000	.	.003	.056	.087	.012	.319
	Age	.244	.097	.003	.	.000	.019	.317	.134
	BMI	.002	.026	.056	.000	.	.159	.248	.365
	IPAQ	.005	.375	.087	.019	.159	.	.091	.034
	MCQ	.253	.488	.012	.317	.248	.091	.	.157
	Iron	.112	.309	.319	.134	.365	.034	.157	.
N	VT_NBS	233	233	233	233	233	223	169	228
	Haemoglobin	233	234	234	234	234	224	170	229
	Ferritin	233	234	234	234	234	224	170	229
	Age	233	234	234	234	234	224	170	229
	BMI	233	234	234	234	234	224	170	229
	IPAQ	223	224	224	224	224	224	163	220

	MCQ	169	170	170	170	170	163	170	167
	Iron	228	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: VT_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.274[a]	.075	.033	8.20089	.075	1.800	7	155	.091	1.783

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: VT_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	847.333	7	121.048	1.800	.091[b]
	Residual	10424.457	155	67.255		
	Total	11271.790	162			

a. Dependent Variable: VT_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

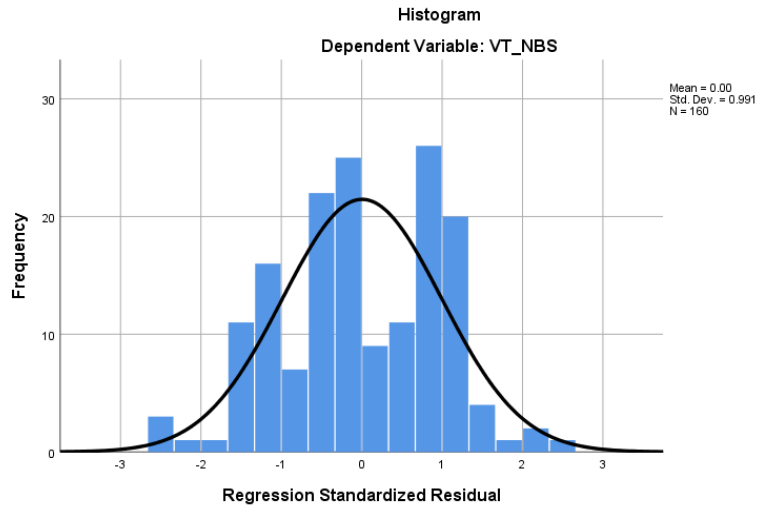
		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	56.021	13.540		4.138	.000					
	Haemoglobin	.001	.104	.001	.010	.992	-.008	.001	.001	.893	1.120
	Ferritin	.017	.022	.065	.780	.437	.039	.063	.060	.861	1.162
	Age	.006	.079	.006	.073	.942	-.046	.006	.006	.864	1.157
	BMI	-.404	.174	-.189	-2.327	.021	-.192	-.184	-.180	.907	1.102
	IPAQ	.001	.000	.163	2.064	.041	.173	.164	.159	.951	1.051
	MCQ	-.185	.277	-.053	-.667	.506	-.051	-.053	-.052	.951	1.051
	Iron	.165	.183	.071	.903	.368	.081	.072	.070	.968	1.033

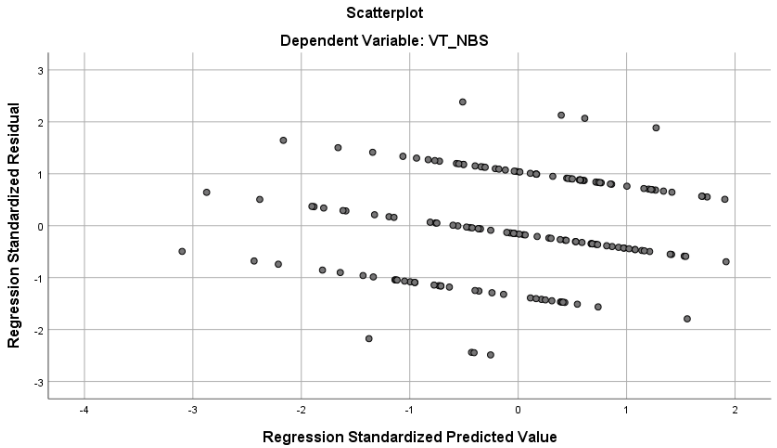
a. Dependent Variable: VT_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: VT_NBS





Social Functioning

Correlations

		SF_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	SF_NBS	1.000	.007	.116	.089	.009	.052	-.083	.077
	Haemoglobin	.007	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.116	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.089	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	.009	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.052	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.083	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.077	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	SF_NBS	.	.457	.039	.090	.446	.221	.141	.126
	Haemoglobin	.457	.	.000	.097	.026	.375	.488	.309
	Ferritin	.039	.000	.	.003	.056	.087	.012	.319
	Age	.090	.097	.003	.	.000	.019	.317	.134
	BMI	.446	.026	.056	.000	.	.159	.248	.365
	IPAQ	.221	.375	.087	.019	.159	.	.091	.034
	MCQ	.141	.488	.012	.317	.248	.091	.	.157
	Iron	.126	.309	.319	.134	.365	.034	.157	.
N	SF_NBS	231	231	231	231	231	222	168	226
	Haemoglobin	231	234	234	234	234	224	170	229
	Ferritin	231	234	234	234	234	224	170	229
	Age	231	234	234	234	234	224	170	229
	BMI	231	234	234	234	234	224	170	229
	IPAQ	222	224	224	224	224	224	163	220

	MCQ	168	170	170	170	170	163	170	167
	Iron	226	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: SF_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.184[a]	.034	-.010	7.81538	.034	.773	7	155	.611	1.975

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: SF_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	330.362	7	47.195	.773	.611[b]
	Residual	9467.437	155	61.080		
	Total	9797.799	162			

a. Dependent Variable: SF_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

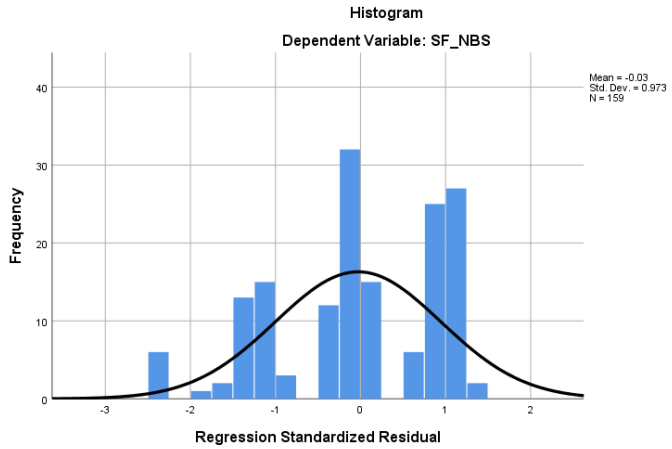
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	46.990	12.903		3.642	.000					
	Haemoglobin	-.015	.099	-.013	-.153	.878	.007	-.012	-.012	.893	1.120
	Ferritin	.025	.021	.103	1.211	.228	.116	.097	.096	.861	1.162
	Age	.065	.076	.073	.864	.389	.089	.069	.068	.864	1.157
	BMI	-.024	.166	-.012	-.143	.887	.009	-.011	-.011	.907	1.102
	IPAQ	.000	.000	.069	.857	.393	.052	.069	.068	.951	1.051
	MCQ	-.246	.264	-.075	-.930	.354	-.083	-.074	-.073	.951	1.051
	Iron	.158	.175	.072	.902	.368	.077	.072	.071	.968	1.033

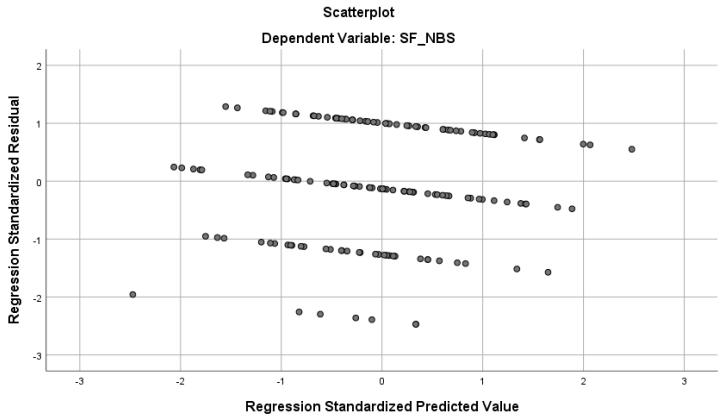
a. Dependent Variable: SF_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: SF_NBS





Role limitations due to emotional functioning

Correlations

		RE_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	RE_NBS	1.000	-.062	.152	.117	.047	.100	-.045	.028
	Haemoglobin	-.062	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.152	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.117	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	.047	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.100	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.045	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.028	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	RE_NBS	.	.172	.010	.037	.237	.067	.280	.336
	Haemoglobin	.172	.	.000	.097	.026	.375	.488	.309
	Ferritin	.010	.000	.	.003	.056	.087	.012	.319
	Age	.037	.097	.003	.	.000	.019	.317	.134
	BMI	.237	.026	.056	.000	.	.159	.248	.365
	IPAQ	.067	.375	.087	.019	.159	.	.091	.034
	MCQ	.280	.488	.012	.317	.248	.091	.	.157
	Iron	.336	.309	.319	.134	.365	.034	.157	.
N	RE_NBS	234	234	234	234	234	224	170	229
	Haemoglobin	234	234	234	234	234	224	170	229
	Ferritin	234	234	234	234	234	224	170	229
	Age	234	234	234	234	234	224	170	229
	BMI	234	234	234	234	234	224	170	229
	IPAQ	224	224	224	224	224	224	163	220

	MCQ	170	170	170	170	170	163	170	167
	Iron	229	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: RE_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.240[a]	.058	.015	10.23955	.058	1.355	7	155	.228	1.828

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: RE_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	994.792	7	142.113	1.355	.228[b]
	Residual	16251.512	155	104.848		
	Total	17246.304	162			

a. Dependent Variable: RE_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

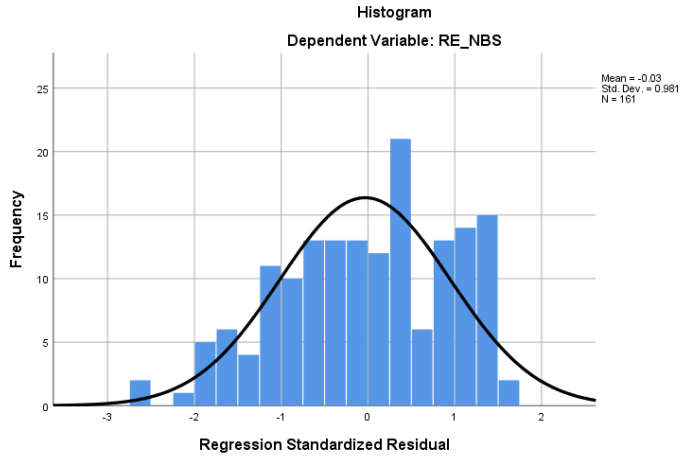
		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	54.301	16.906		3.212	.002					
	Haemoglobin	-.156	.130	-.100	-1.206	.230	-.062	-.096	-.094	.893	1.120
	Ferritin	.054	.027	.166	1.980	.049	.152	.157	.154	.861	1.162
	Age	.102	.099	.086	1.027	.306	.117	.082	.080	.864	1.157
	BMI	.080	.217	.030	.368	.713	.047	.030	.029	.907	1.102
	IPAQ	.001	.000	.128	1.605	.111	.100	.128	.125	.951	1.051
	MCQ	-.128	.346	-.030	-.370	.712	-.045	-.030	-.029	.951	1.051
	Iron	.046	.229	.016	.203	.839	.028	.016	.016	.968	1.033

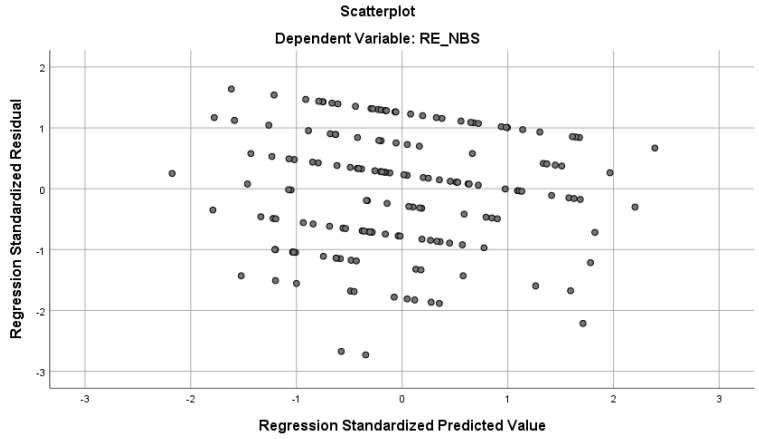
a. Dependent Variable: RE_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: RE_NBS





Mental Health

Correlations

		MH_NBS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	MH_NBS	1.000	.001	.143	.067	-.042	.081	-.120	.026
	Haemoglobin	.001	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.143	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.067	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.042	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.081	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.120	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.026	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	MH_NBS	.	.492	.015	.153	.260	.114	.060	.346
	Haemoglobin	.492	.	.000	.097	.026	.375	.488	.309
	Ferritin	.015	.000	.	.003	.056	.087	.012	.319
	Age	.153	.097	.003	.	.000	.019	.317	.134
	BMI	.260	.026	.056	.000	.	.159	.248	.365
	IPAQ	.114	.375	.087	.019	.159	.	.091	.034
	MCQ	.060	.488	.012	.317	.248	.091	.	.157
	Iron	.346	.309	.319	.134	.365	.034	.157	.
N	MH_NBS	233	233	233	233	233	223	169	228
	Haemoglobin	233	234	234	234	234	224	170	229
	Ferritin	233	234	234	234	234	224	170	229
	Age	233	234	234	234	234	224	170	229
	BMI	233	234	234	234	234	224	170	229
	IPAQ	223	224	224	224	224	224	163	220

	MCQ	169	170	170	170	170	163	170	167
	Iron	228	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: MH_NBS

b. All requested variables entered.

Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.219[a]	.048	.005	9.11021	.048	1.111	7	155	.359	1.893

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: MH_NBS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	645.272	7	92.182	1.111	.359[b]
	Residual	12864.362	155	82.996		
	Total	13509.634	162			

a. Dependent Variable: MH_NBS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

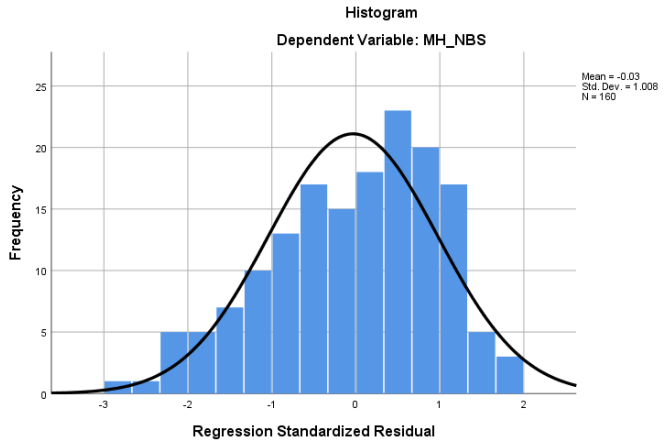
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	46.788	15.041		3.111	.002					
	Haemoglobin	-.027	.115	-.019	-.230	.818	.001	-.018	-.018	.893	1.120
	Ferritin	.039	.024	.134	1.590	.114	.143	.127	.125	.861	1.162
	Age	.068	.088	.065	.774	.440	.067	.062	.061	.864	1.157
	BMI	-.137	.193	-.059	-.711	.478	-.042	-.057	-.056	.907	1.102
	IPAQ	.000	.000	.106	1.321	.188	.081	.106	.104	.951	1.051
	MCQ	-.400	.308	-.104	-1.298	.196	-.120	-.104	-.102	.951	1.051
	Iron	.059	.204	.023	.288	.774	.026	.023	.023	.968	1.033

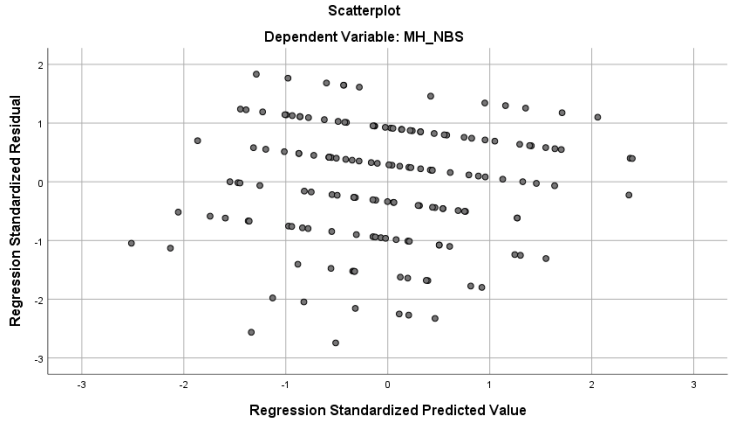
a. Dependent Variable: MH_NBS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: MH_NBS





PCS

Correlations

		PCS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	PCS	1.000	-.015	-.120	-.074	-.108	.081	-.024	.047
	Haemoglobin	-.015	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	-.120	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	-.074	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	-.108	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.081	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.024	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.047	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	PCS	.	.411	.035	.133	.052	.116	.381	.240
	Haemoglobin	.411	.	.000	.097	.026	.375	.488	.309
	Ferritin	.035	.000	.	.003	.056	.087	.012	.319
	Age	.133	.097	.003	.	.000	.019	.317	.134
	BMI	.052	.026	.056	.000	.	.159	.248	.365
	IPAQ	.116	.375	.087	.019	.159	.	.091	.034
	MCQ	.381	.488	.012	.317	.248	.091	.	.157
	Iron	.240	.309	.319	.134	.365	.034	.157	.
N	PCS	229	229	229	229	229	220	167	224
	Haemoglobin	229	234	234	234	234	224	170	229
	Ferritin	229	234	234	234	234	224	170	229
	Age	229	234	234	234	234	224	170	229
	BMI	229	234	234	234	234	224	170	229
	IPAQ	220	224	224	224	224	224	163	220

	MCQ	167	170	170	170	170	163	170	167
	Iron	224	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: PCS

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.181[a]	.033	-.011	5.62508	.033	.749	7	155	.631	1.894

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: PCS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	165.834	7	23.691	.749	.631[b]
	Residual	4904.428	155	31.641		
	Total	5070.262	162			

a. Dependent Variable: PCS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

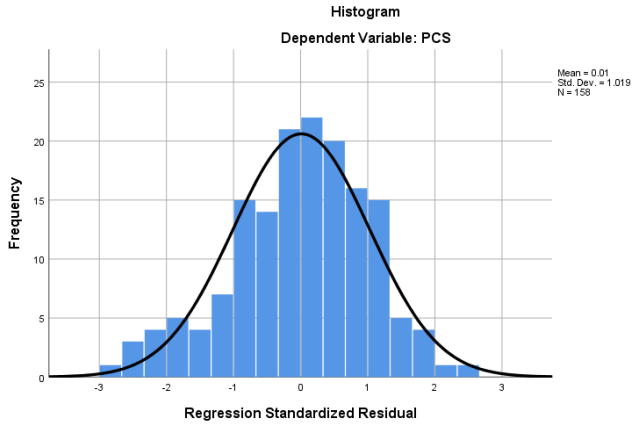
Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	57.948	9.287		6.240	.000					
	Haemoglobin	.020	.071	.023	.275	.783	-.015	.022	.022	.893	1.120
	Ferritin	-.020	.015	-.113	-1.333	.185	-.120	-.106	-.105	.861	1.162
	Age	-.016	.054	-.026	-.303	.762	-.074	-.024	-.024	.864	1.157
	BMI	-.125	.119	-.087	-1.045	.297	-.108	-.084	-.083	.907	1.102
	IPAQ	.000	.000	.062	.760	.448	.081	.061	.060	.951	1.051
	MCQ	-.116	.190	-.049	-.609	.543	-.024	-.049	-.048	.951	1.051
	Iron	.068	.126	.043	.540	.590	.047	.043	.043	.968	1.033

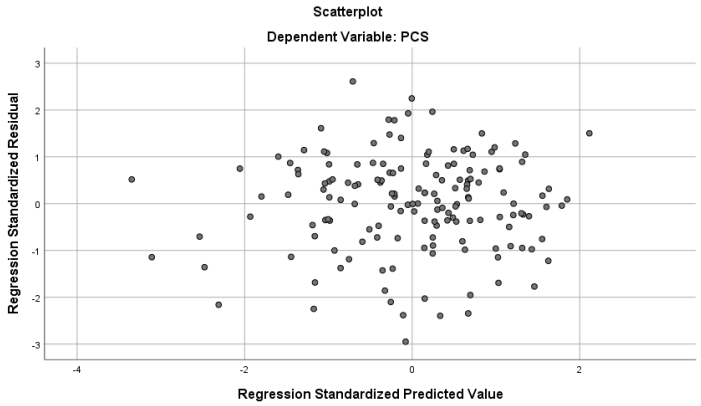
a. Dependent Variable: PCS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: PCS





MCS

Correlations

		MCS	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
Pearson Correlation	MCS	1.000	.010	.160	.104	.028	.100	-.072	.046
	Haemoglobin	.010	1.000	.258	-.085	.127	-.021	-.002	.033
	Ferritin	.160	.258	1.000	.182	.104	-.091	-.173	-.031
	Age	.104	-.085	.182	1.000	.254	-.139	-.037	.074
	BMI	.028	.127	.104	.254	1.000	-.067	.053	.023
	IPAQ	.100	-.021	-.091	-.139	-.067	1.000	.105	.123
	MCQ	-.072	-.002	-.173	-.037	.053	.105	1.000	.078
	Iron	.046	.033	-.031	.074	.023	.123	.078	1.000
Sig. (1-tailed)	MCS	.	.441	.008	.059	.338	.070	.180	.248
	Haemoglobin	.441	.	.000	.097	.026	.375	.488	.309
	Ferritin	.008	.000	.	.003	.056	.087	.012	.319
	Age	.059	.097	.003	.	.000	.019	.317	.134
	BMI	.338	.026	.056	.000	.	.159	.248	.365
	IPAQ	.070	.375	.087	.019	.159	.	.091	.034
	MCQ	.180	.488	.012	.317	.248	.091	.	.157
	Iron	.248	.309	.319	.134	.365	.034	.157	.
N	MCS	229	229	229	229	229	220	166	224
	Haemoglobin	229	234	234	234	234	224	170	229
	Ferritin	229	234	234	234	234	224	170	229
	Age	229	234	234	234	234	224	170	229
	BMI	229	234	234	234	234	224	170	229
	IPAQ	220	224	224	224	224	224	163	220

	MCQ	166	170	170	170	170	163	170	167
	Iron	224	229	229	229	229	220	167	229

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin[b]	.	Enter

a. Dependent Variable: MCS

b. All requested variables entered.

Model Summary[b]

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	.227[a]	.052	.009	9.54926	.052	1.204	7	155	.304	1.865

a. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

b. Dependent Variable: MCS

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	768.470	7	109.781	1.204	.304[b]
	Residual	14134.205	155	91.188		
	Total	14902.676	162			

a. Dependent Variable: MCS

b. Predictors: (Constant), Iron, BMI, MCQ, Haemoglobin, IPAQ, Age, Ferritin

Coefficients[a]

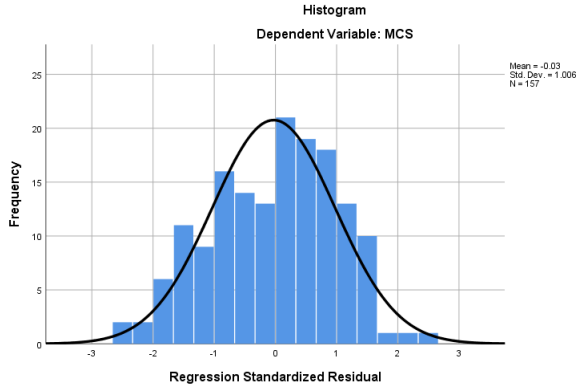
		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	40.218	15.766		2.551	.012					
	Haemoglobin	-.030	.121	-.021	-.252	.802	.010	-.020	-.020	.893	1.120
	Ferritin	.046	.026	.152	1.799	.074	.160	.143	.141	.861	1.162
	Age	.095	.092	.087	1.029	.305	.104	.082	.081	.864	1.157
	BMI	.009	.202	.004	.043	.966	.028	.003	.003	.907	1.102
	IPAQ	.001	.000	.127	1.588	.114	.100	.126	.124	.951	1.051
	MCQ	-.235	.323	-.058	-.729	.467	-.072	-.058	-.057	.951	1.051
	Iron	.091	.213	.034	.424	.672	.046	.034	.033	.968	1.033

a. Dependent Variable: MCS

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions							
				(Constant)	Haemoglobin	Ferritin	Age	BMI	IPAQ	MCQ	Iron
1	1	6.937	1.000	.00	.00	.00	.00	.00	.00	.00	.00
	2	.418	4.074	.00	.00	.38	.00	.00	.08	.25	.00
	3	.283	4.954	.00	.00	.00	.00	.00	.66	.38	.00
	4	.204	5.835	.00	.00	.51	.04	.01	.16	.35	.04
	5	.084	9.076	.00	.00	.03	.25	.01	.04	.00	.77
	6	.057	10.984	.01	.01	.01	.64	.05	.04	.02	.16
	7	.015	21.262	.03	.03	.00	.02	.93	.01	.00	.02
	8	.001	76.520	.97	.97	.06	.04	.00	.00	.00	.00

a. Dependent Variable: MCS



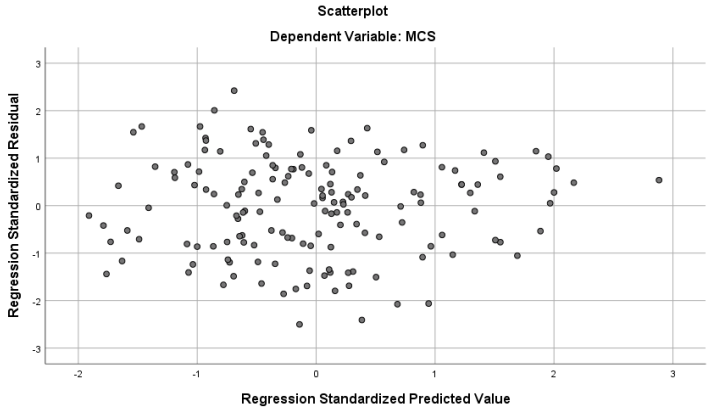


Table 0.1 Cognitive task analysis outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Episodic Accuracy	Haemoglobin	.003	-.045	.511
	Serum ferritin		-.025	.708
Episodic Speed	Haemoglobin	.004	-.001	.992
	Serum ferritin		-.067	.328
Working Memory Accuracy	Haemoglobin	.047	.094	.162
	Serum ferritin		-.096	.154
	Years in education		.199	.003
Working Memory Speed	Haemoglobin	.005	.001	.988
	Serum ferritin		-.068	.320
Executive Function Accuracy	Haemoglobin	.010	.100	.141
	Serum ferritin		-.007	.921
Executive Function Speed	Haemoglobin	.064	.062	.351
	Serum ferritin		-.012	.858
	Age		.254	.000
Sustained Attention Accuracy	Haemoglobin	.044	.039	.558
	Serum ferritin		-.057	.406
	Age		.156	.020
Sustained Attention Speed	Haemoglobin	.001	.025	.712
	Serum ferritin		-.019	.781
Learning	Haemoglobin	.040	.088	.198
	Serum ferritin		-.036	.599
	BMI		-.189	.004

Table 0.2 Subjective mood analysis (POMS) outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Tension-Anxiety	Haemoglobin	.008	.095	.166
	Serum ferritin		-.026	.707
Depression-Dejection	Haemoglobin	.001	-.032	.651
	Serum ferritin		.032	.650
Anger-Hostility	Haemoglobin	.000	.017	.808
	Serum ferritin		-.007	.920
Vigour-Activity	Haemoglobin	.010	-.030	.657
	Serum ferritin		.103	.134
Fatigue-Inertia	Haemoglobin	.011	.109	.111
	Serum ferritin		-.025	.714
Confusion-Bewilderment	Haemoglobin	.008	.077	.262
	Serum ferritin		-.075	.278
Total Mood Disturbance	Haemoglobin	.008	.094	.195

	Serum ferritin		-.033	.645

Table 0.3 Subjective mood analysis (PSS and SCI) outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Perceived Stress	Haemoglobin	.051	.124	.116
	Serum ferritin		-.111	.163
	Menstrual blood loss		.157	.043
Sleep Quality	Haemoglobin	.079	-.027	.725
	Serum ferritin		.021	.789
	Menstrual blood loss		-.275	.000

Table 0.4 Subjective workload analysis (NASA-TLX) outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Total Workload	Haemoglobin	.012	.113	.099
	Serum ferritin		-.035	.614

Table 0.5 Subjective fatigue analysis (PFS and VAS) outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Behavioural-Severity	Haemoglobin	.038	.083	.292
	Serum ferritin		-.044	.583
	Menstrual blood loss		.168	.031
Affective Meaning	Haemoglobin	.045	.096	.223
	Serum ferritin		-.056	.480
	Menstrual blood loss		.179	.022
Sensory	Haemoglobin	.020	.131	.053
	Serum ferritin		-.095	.161
Cognition-Mood	Haemoglobin	.008	.084	.215
	Serum ferritin		-.063	.356
Total Fatigue	Haemoglobin	.016	.115	.090
	Serum ferritin		-.090	.183
Mental Fatigue	Haemoglobin	.009	.066	.329
	Serum ferritin		-.090	.184
Alertness	Haemoglobin	.027	-.042	.597
	Serum ferritin		.034	.672
	Menstrual blood loss		-.151	.054

Table 0.6 Subjective wellbeing analysis (SF-12) outcomes for haemoglobin and serum ferritin. R^2, β-weightings and p values of the unadjusted or adjusted models for haemoglobin and serum ferritin.

		R^2	β	p
Physical Functioning	Haemoglobin	.015	-.100	.142
	Serum ferritin		-.049	.470
Role-Physical	Haemoglobin	.007	-.044	.519
	Serum ferritin		.081	.234
Bodily Pain	Haemoglobin	.026	-.111	.099
	Serum ferritin		-.091	.178
General Health	Haemoglobin	.001	.017	.799
	Serum ferritin		-.035	.605
Vitality	Haemoglobin	.068	.001	.991
	Serum ferritin		.073	.283
	BMI		-.189	.005
	Physical activity		.167	.012
Social Functioning	Haemoglobin	.014	-.025	.719
	Serum ferritin		.123	.073
Role-Emotional	Haemoglobin	.034	-.109	.105
	Serum ferritin		.181	.008
Mental Health	Haemoglobin	.022	-.038	.573
	Serum ferritin		.153	.025
PCS	Haemoglobin	.015	.017	.802
	Serum ferritin		-.124	.071
MCS	Haemoglobin	.027	-.034	.621
	Serum ferritin		.169	.014

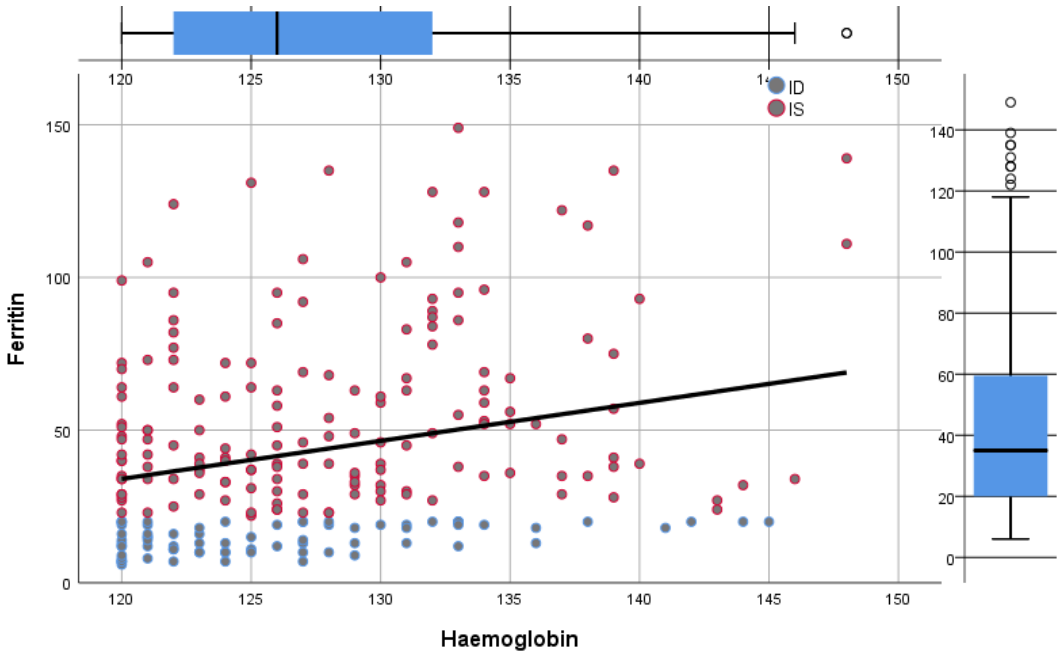


Figure 0.1 The correlation of haemoglobin and serum ferritin grouped by iron status

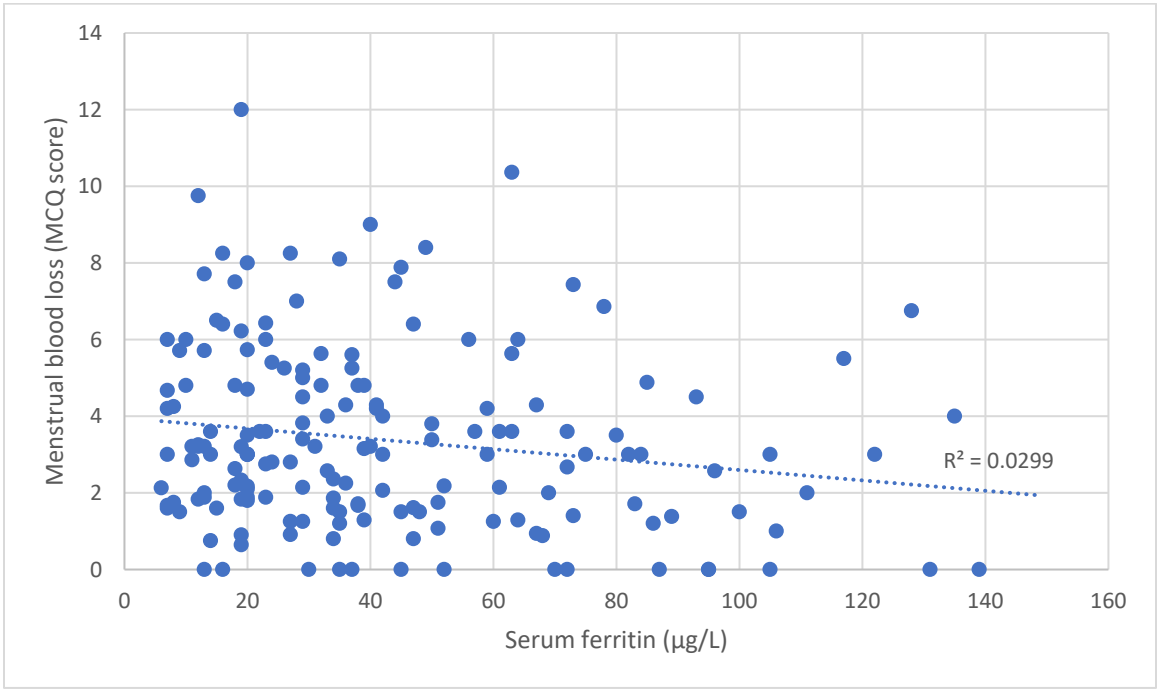


Figure 0.2 The correlation of serum ferritin and menstrual blood loss

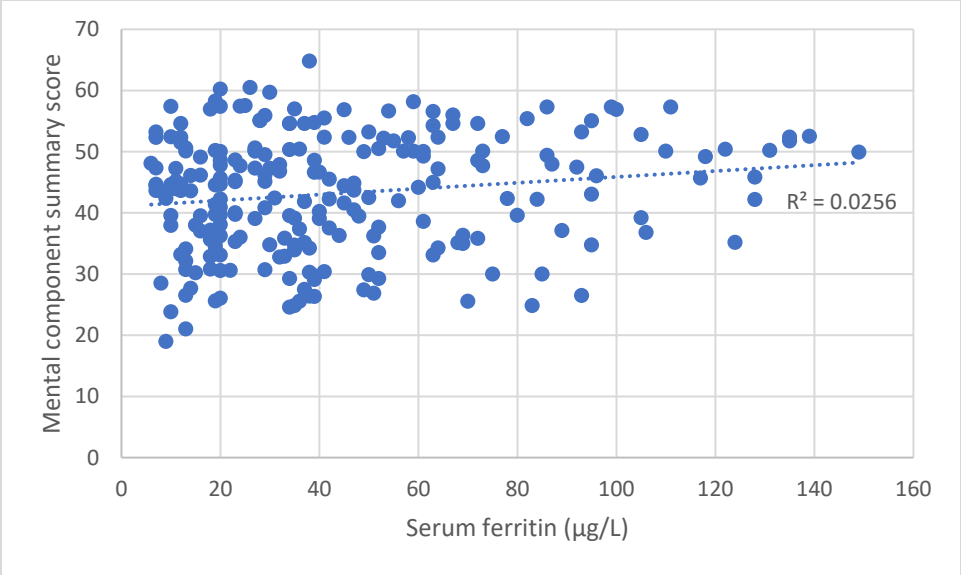


Figure 0.3 The correlation of serum ferritin and MCS scores

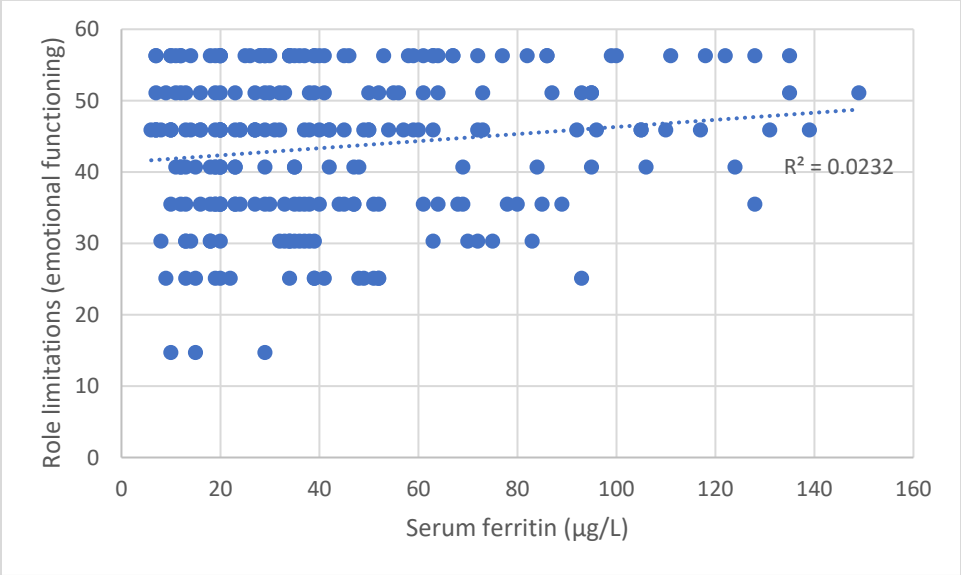


Figure 0.4 The correlation of serum ferritin and role limitations due to emotional functioning

APPENDIX V: Participant disposition throughout the thesis

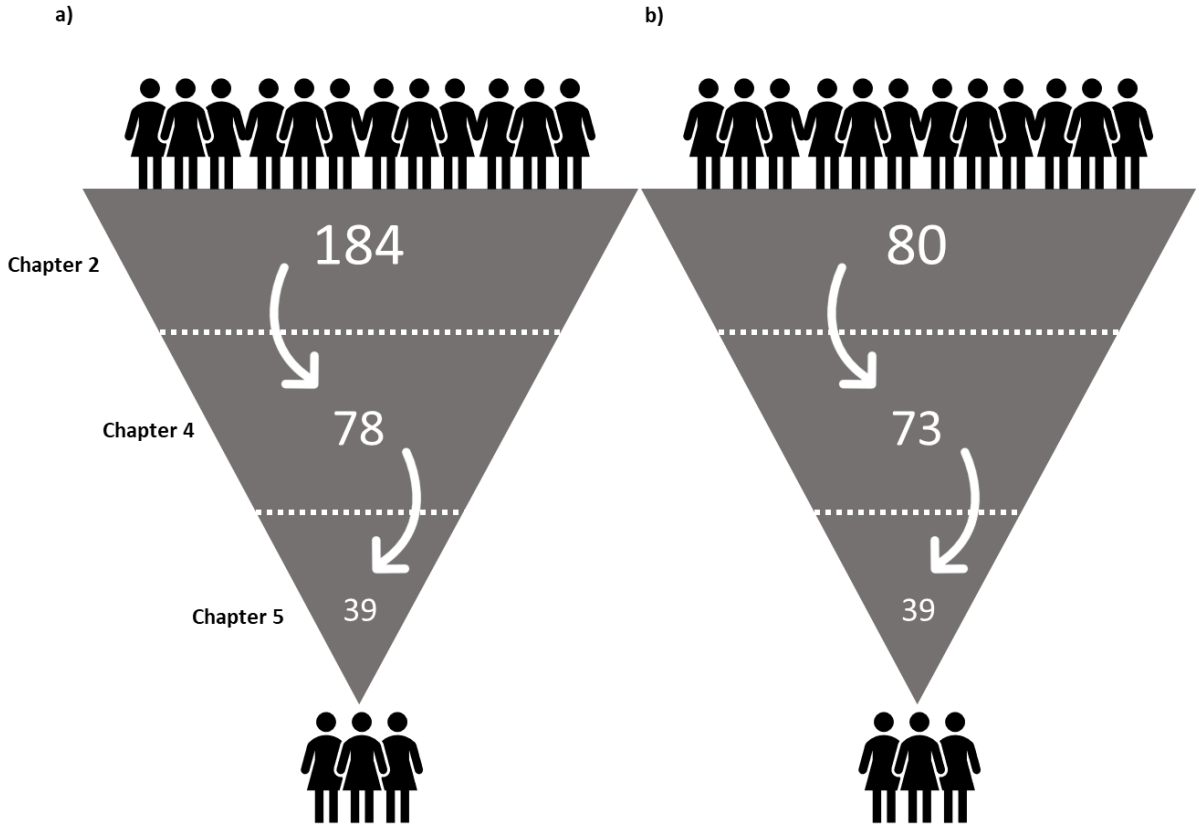


Figure 0.5 Disposition of enrolled participants throughout the thesis for a) number of iron sufficient women and b) number of non-anaemic iron deficient women. All participants enrolled in the study presented in Chapter 4 had been enrolled in the study presented in Chapter 2. All participants enrolled in the study presented in Chapter 5 had been enrolled in the studies presented in Chapters 2 and 4.

APPENDIX VI: Treatment guess questionnaire

Study Code: 9BN1 Date: ____/____/____

Subject ID:

Randomisation No.:

Visit:

Which treatment do you think you were administered? (please circle)

1. Placebo (dummy pill)
2. Active (iron)

What is your reason(s) for thinking this?

_____

_____

_____

APPENDIX VII: Treatment diary

SUBJECT DIARY

9BN1 The effects of daily iron and iron + vitamin C supplementation on brain function, subjective mood and fatigue in women of reproductive age following a 16-week intervention

Subject ID: |__ |__ |__|

Random Number: |__ |__ |__|

Date Subject Diary dispensed: |__ |__ |-|__ |__ |-|__ |__| *(dd-mm-yy)*

Next Appointment: _____

Dear Participant,

For the success of the study it is very important that you follow the instructions of the study team very carefully. Therefore, please find a short summary of the most important points.

Treatment consumption instructions
Take 1 tablet per day, immediately upon wakening. Take tablets with water. Do not exceed the recommended dose.

Diet
During the course of the whole study you should not change your dietary habits.

Medication / Therapy or Health Problems or Symptoms
If you take any new medication or therapy or if you have any health problems or unusual symptoms you should document in as much detail as possible in this diary on the appropriate pages and talk about it to the researcher during the next appointment. You do not need to write down routine medications which have already been discussed at the screening visit.

In general, you should avoid, if possible, the use of non-prescription drugs during the study. However, if you intend to use non-prescription drugs within the week preceding the next appointment, **please contact the research team**. There is a chance that the visit might be postponed.

On the morning of your last appointment, do not take your treatment.

Please bring this diary and any unused tablets to the next appointment.

If you have any questions or any problems occur, please contact:

Name: Hannah Avery
E-mail: hannah.l.avery@northumbria.ac.uk

Or any member of the research team on 0191 243 7252 (office hours).

Please remember to take your treatment immediately upon wakening. In the table below, please record the date and time each day that take the treatment. If you forget to take it, please leave this box blank. Any ill health or intake of non-routine medications/therapy should be recorded on pages 5-8.

Week 1		Week 2		Week 3		Week 4	
Date	***Time***	***Date***	***Time***	***Date***	***Time***	***Date***	***Time***

Please remember to take your treatment immediately upon wakening. In the table below, please record the date and time each day that take the treatment. If you forget to take it, please leave this box blank. Any ill health or intake of non-routine medications/therapy should be recorded on pages 5-8.

Week 5		Week 6		Week 7		Week 8	
Date	***Time***	***Date***	***Time***	***Date***	***Time***	***Date***	***Time***

Have you taken any medication or dietary supplements?

☐ No
☐ Yes: *please give details in the table below. Please include the product/drug/therapy name in full, the amount (e.g. 1x200mg tablet), the date you took it and what you took it for.*

Product/drug/therapy name	**Quantity**	**Dose**	**Date**	**What you took it for?**
Example: paracetamol	*2 / day*	*500 mg/tablet*	*25-Apr-12*	*headache*

Product/drug/therapy name	**Quantity**	**Dose**	**Date**	**What you took it for?**
Example: paracetamol	*2 / day*	*500 mg/tablet*	*25-Apr-12*	*headache*

Have you experienced any new health problems or unusual symptoms?

☐ No
☐ Yes*, please give details in the table below. Please include your symptom(s), the date(s) it/they started and stopped if applicable, its/their severity and any action you took to relieve it/them.*

Symptom	**Date symptom started**	**Please rate the severity of the symptom in terms of how it affects your daily functioning 1: Mild 2: Moderate 3: Severe**	**If resolved please give the date it stopped**	**Did you do anything to relieve your health problems/symptoms (like drug, therapy)?**
Example: headache	*25-Apr-12*	*moderate*	*25-Apr-12*	*paracetamol*

Symptom	Date symptom started	Please rate the severity of the symptom in terms of how it affects your daily functioning 1: Mild 2: Moderate 3: Severe	If resolved please give the date it stopped	Did you do anything to relieve your health problems/symptoms (like drug, therapy)?
Example: headache	*25-Apr-12*	*moderate*	*25-Apr-12*	*paracetamol*

APPENDIX VIII: Chapter 4 cognitive outcomes

Mixed Model Analysis – Episodic Memory Accuracy

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Episodic_Accuracy	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Episodic_Accuracy.

Information Criteria[a]

-2 Restricted Log Likelihood	217.157
Akaike's Information Criterion (AIC)	219.157
Hurvich and Tsai's Criterion (AICC)	219.193
Bozdogan's Criterion (CAIC)	222.893
Schwarz's Bayesian Criterion (BIC)	221.893

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Episodic_Accuracy.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	114	.055	.814
Treatment	2	114	.782	.460
Baseline_Episodic_Accuracy	1	114	29.951	.000
Baseline_sft	1	114	.206	.651

a. Dependent Variable: PD_Episodic_Accuracy.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.311902	.041312

a. Dependent Variable: PD_Episodic_Accuracy.

Estimated Marginal Means

Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
1	-.090[b]	.091	114	-.271	.091
2	.065[b]	.087	114	-.107	.236
3	-.036[b]	.090	114	-.213	.142

a. Dependent Variable: PD_Episodic_Accuracy.

b. Covariates appearing in the model are evaluated at the following values: Baseline_Episodic_Accuracy = -.0249, Baseline_sft = 34.50.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b]
						Lower Bound
1	3	-.054	.128	114	.893	-.345
2	3	.100	.125	114	.667	-.182

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	114	.782	.460

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_Episodic_Accuracy.

Mixed Model Analysis – Episodic Speed

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Episodic_Speed	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Episodic_Speed.

Information Criteria[a]

-2 Restricted Log Likelihood	224.437
Akaike's Information Criterion (AIC)	226.437
Hurvich and Tsai's Criterion (AICC)	226.474
Bozdogan's Criterion (CAIC)	230.137
Schwarz's Bayesian Criterion (BIC)	229.137

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Episodic_Speed.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	110	1.287	.259
Treatment	2	110.000	.683	.507
Baseline_Episodic_Speed	1	110	94.702	.000
Baseline_sft	1	110	.237	.627

a. Dependent Variable: PD_Episodic_Speed.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.352986	.047597

a. Dependent Variable:
PD_Episodic_Speed.

Estimated Marginal Means

Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
1	.038[b]	.099	110	-.158	.235
2	-.120[b]	.093	110	-.305	.064
3	-.048[b]	.097	110	-.240	.144

a. Dependent Variable: PD_Episodic_Speed.
b. Covariates appearing in the model are evaluated at the following values:
Baseline_Episodic_Speed = .0416, Baseline_sft = 34.10.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b]
						Lower Bound
1	3	.086	.139	110	.783	-.228
2	3	-.072	.135	110	.835	-.378

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	110.000	.683	.507

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]
a. Dependent Variable: PD_Episodic_Speed.

Mixed Model Analysis – Executive Function Accuracy

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Exec_Func_Accuracy	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Exec_Func_Accuracy.

Information Criteria[a]

-2 Restricted Log Likelihood	234.025
Akaike's Information Criterion (AIC)	236.025
Hurvich and Tsai's Criterion (AICC)	236.061
Bozdogan's Criterion (CAIC)	239.761
Schwarz's Bayesian Criterion (BIC)	238.761

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Exec_Func_Accuracy.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	114	1.460	.229
Treatment	2	114	.522	.595
Baseline_Exec_Func_Accuracy	1	114	24.298	.000
Baseline_sft	1	114	2.634	.107

a. Dependent Variable: PD_Exec_Func_Accuracy.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.362800	.048054

a. Dependent Variable: PD_Exec_Func_Accuracy.

Estimated Marginal Means

Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
1	.041[b]	.098	114	-.153	.235
2	-.075[b]	.093	114	-.259	.110
3	.047[b]	.097	114	-.145	.238

a. Dependent Variable: PD_Exec_Func_Accuracy.

b. Covariates appearing in the model are evaluated at the following values: Baseline_Executive_Function_Accuracy = -.0054, Baseline_sft = 34.50.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	
1	3	-.006	.138	114	.999	-.317	
2	3	-.121	.135	114	.602	-.427	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	114	.522	.595

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_Exec_Func_Accuracy.

Mixed Model Analysis – Executive Function Speed

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Exec_Func_Speed	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Exec_Func_Speed.

Information Criteria[a]

-2 Restricted Log Likelihood	220.741
Akaike's Information Criterion (AIC)	222.741
Hurvich and Tsai's Criterion (AICC)	222.777
Bozdogan's Criterion (CAIC)	226.477
Schwarz's Bayesian Criterion (BIC)	225.477

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Exec_Func_Speed.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	114	.829	.365
Treatment	2	114	.735	.482
Baseline_Exec_Func_Speed	1	114	114.046	.000
Baseline_sft	1	114	1.663	.200

a. Dependent Variable: PD_Exec_Func_Speed.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.321576	.042594

a. Dependent Variable: PD_Exec_Func_Speed.

Estimated Marginal Means

Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
1	.063[b]	.092	114	-.120	.246
2	-.070[b]	.088	114	-.244	.104
3	.063[b]	.091	114	-.118	.243

a. Dependent Variable: PD_Exec_Func_Speed.

b. Covariates appearing in the model are evaluated at the following values: Baseline_Executive_Function_Speed = .0120, Baseline_sft = 34.50.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	
1	3	-8.961E-5	.130	114	1.000	-.294	
2	3	-.133	.127	114	.506	-.420	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	114	.735	.482

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_Exec_Func_Speed.

Mixed Model Analysis – Attention Accuracy

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Attention_Accuracy	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Attention_Acc.

Information Criteria[a]

-2 Restricted Log Likelihood	272.512
Akaike's Information Criterion (AIC)	274.512
Hurvich and Tsai's Criterion (AICC)	274.548
Bozdogan's Criterion (CAIC)	278.239
Schwarz's Bayesian Criterion (BIC)	277.239

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Attention_Acc.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	113	.070	.791
Treatment	2	113	1.581	.210
Baseline_Attention_Accuracy	1	113	83.356	.000
Baseline_sft	1	113	.011	.918

a. Dependent Variable: PD_Attention_Acc.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.515097	.068527

a. Dependent Variable: PD_Attention_Acc.

Estimated Marginal Means

Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
1	.001[b]	.118	113	-.233	.235
2	.086[b]	.111	113	-.134	.306
3	-.194[b]	.115	113	-.422	.035

a. Dependent Variable: PD_Attention_Acc.

b. Covariates appearing in the model are evaluated at the following values: Baseline_Attention_Accuracy = -.0219, Baseline_sft = 34.45.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	Lower Bound	
1	3	.195	.165	113	.423	-.179	
2	3	.280	.161	113	.161	-.084	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	113	1.581	.210

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_Attention_Acc.

Mixed Model Analysis – Attention Speed

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_Attention_Speed	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_Attention_Speed.

Information Criteria[a]

-2 Restricted Log Likelihood	197.013
Akaike's Information Criterion (AIC)	199.013
Hurvich and Tsai's Criterion (AICC)	199.049
Bozdogan's Criterion (CAIC)	202.741
Schwarz's Bayesian Criterion (BIC)	201.741

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_Attention_Speed.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	113	.176	.676
Treatment	2	113	2.120	.125
Baseline_Attention_Speed	1	113	209.242	.000
Baseline_sft	1	113	.678	.412

a. Dependent Variable: PD_Attention_Speed.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.263957	.035116

a. Dependent Variable: PD_Attention_Speed.

Estimated Marginal Means

Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
1	-.106[b]	.085	113	-.274	.062
2	.092[b]	.079	113	-.066	.249
3	.116[b]	.083	113	-.047	.280

a. Dependent Variable: PD_Attention_Speed.

b. Covariates appearing in the model are evaluated at the following values: Baseline_Attention_Speed = .0205, Baseline_sft = 34.45.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	Lower Bound	
1	3	-.222	.118	113	.122	-.490	
2	3	-.024	.115	113	.972	-.285	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	113	2.120	.125

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_Attention_Speed.

Mixed Model Analysis – Learning (CLLT)

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_CLLT	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_CLLT.

Information Criteria[a]

-2 Restricted Log Likelihood	235.236
Akaike's Information Criterion (AIC)	237.236
Hurvich and Tsai's Criterion (AICC)	237.275
Bozdogan's Criterion (CAIC)	240.890
Schwarz's Bayesian Criterion (BIC)	239.890

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_CLLT.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	105	.225	.637
Treatment	2	105	2.494	.087
Baseline_CLLT	1	105	.058	.811
Baseline_sft	1	105	.084	.773

a. Dependent Variable: PD_CLLT.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.429299	.059249

a. Dependent Variable: PD_CLLT.

Estimated Marginal Means

Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
1	-.181[b]	.114	105	-.407	.045
2	.154[b]	.109	105	-.062	.370
3	.102[b]	.107	105	-.111	.314

a. Dependent Variable: PD_CLLT.

b. Covariates appearing in the model are evaluated at the following values: Baseline_CLLT = -.0022, Baseline_sft = 34.83.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	Lower Bound	
1	3	-.283	.158	105	.148	-.642	
2	3	.052	.152	105	.928	-.293	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	105	2.494	.087

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_CLLT.

Mixed Model Analysis – Working Memory Speed

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_WM_Speed	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD WM Speed.

Information Criteria[a]

-2 Restricted Log Likelihood	226.316
Akaike's Information Criterion (AIC)	228.316
Hurvich and Tsai's Criterion (AICC)	228.358
Bozdogan's Criterion (CAIC)	231.911
Schwarz's Bayesian Criterion (BIC)	230.911

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD WM Speed.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	99	.166	.685
Treatment	2	99	.079	.924
Baseline_WM_Speed	1	99	118.963	.000
Baseline_sft	1	99	.002	.962

a. Dependent Variable: PD WM Speed.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.441554	.062760

a. Dependent Variable: PD WM Speed.

Estimated Marginal Means

Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
1	.009[b]	.122	99	-.232	.250
2	-.042[b]	.108	99	-.257	.173
3	-.054[b]	.112	99	-.277	.169

a. Dependent Variable: PD WM Speed.

b. Covariates appearing in the model are evaluated at the following values: Baseline WM Speed = -.1018, Baseline_sft = 33.99.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b]
						Lower Bound
1	3	.063	.166	99	.914	-.315
2	3	.012	.157	99	.996	-.345

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	99	.079	.924

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD WM Speed.

Mixed Model Analysis – Working Memory Accuracy

Model Dimension[a]

		Number of Levels	Number of Parameters
Fixed Effects	Intercept	1	1
	Treatment	3	2
	Baseline_WM_Acc	1	1
	Baseline_sft	1	1
Residual			1
Total		6	6

a. Dependent Variable: PD_WM_Acc.

Information Criteria[a]

-2 Restricted Log Likelihood	241.746
Akaike's Information Criterion (AIC)	243.746
Hurvich and Tsai's Criterion (AICC)	243.782
Bozdogan's Criterion (CAIC)	247.473
Schwarz's Bayesian Criterion (BIC)	246.473

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_WM_Acc.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	113	2.445	.121
Treatment	2	113	.424	.655
Baseline_WM_Acc	1	113	74.254	.000
Baseline_sft	1	113	2.696	.103

a. Dependent Variable: PD_WM_Acc.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter	Estimate	Std. Error
Residual	.393058	.052292

a. Dependent Variable: PD_WM_Acc.

Estimated Marginal Means

Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
1	-.007[b]	.103	113	-.210	.196
2	.015[b]	.099	113	-.180	.211
3	-.107[b]	.101	113	-.307	.092

a. Dependent Variable: PD_WM_Acc.

b. Covariates appearing in the model are evaluated at the following values: Baseline_WM_Acc = -.0113, Baseline_sft = 34.67.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	Lower Bound	
1	3	.100	.144	113	.738	-.226	
2	3	.122	.141	113	.624	-.197	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	113	.424	.655

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_WM_Acc.

APPENDIX IX: Chapter 4 treatment comparisons for iron status parameters

Table 0.7 Biochemical analysis outcomes for placebo, iron and iron and vitamin C treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of the main treatment effects from the linear mixed models

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	*F*	*p*
Haemoglobin (g/L)	Placebo	39	126.92	1.04	**127.15**	**1.64**		
	Iron	38	127.74	1.04	132.45^{T}	1.67	**3.95**	**.022**
	Iron + Vit C	42	125.10	0.98	**133.07***	**1.60**		
Serum ferritin (µg/L)	Placebo	37	37.90	4.94	**38.80**	**4.39**		
	Iron	37	34.61	4.67	**54.69***	**4.25**	**9.19**	**< .001**
	Iron + Vit C	39	31.26	3.42	**59.00****	**3.62**		

* significant difference between placebo group and an active treatment group below p < .05; ** significant difference between placebo group and an active treatment group below p < .001; ^{T} trend towards a significant difference between placebo group and an active treatment group below p < .10.

A significant effect of treatment for haemoglobin was identified [F (2, 114) = 3.95, p = .022]. Post hoc analyses revealed no significant differences between the iron group (132.45) and the iron and vitamin C group (133.07; p = .075).

A significant effect of treatment for serum ferritin was also identified [F (2, 109) = 9.19, p < .001]. Post hoc analyses revealed no significant differences between the iron group (54.69) and the iron and vitamin C group (59.00; p = .764).

Mixed Model Analysis – Serial Subtractions Total (3's)

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_SS3_Total	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	892.845
Akaike's Information Criterion (AIC)	896.845
Hurvich and Tsai's Criterion (AICC)	896.923
Bozdogan's Criterion (CAIC)	904.945
Schwarz's Bayesian Criterion (BIC)	902.945

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_SS3_Total.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	54.496	15.871	.000
Treatment	2	32.681	1.828	.177
Rep	2	80.363	.916	.404
Treatment * Rep	4	77.486	.543	.705
Baseline_SS3_Total	1	67.979	173.318	.000
Baseline_sft	1	35.762	2.698	.109

a. Dependent Variable: PD_SS3_Total.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	9.824917	1.627809
Intercept [subject = Participant]	Variance	4.816936	2.345204

a. Dependent Variable: PD_SS3_Total.

Estimated Marginal Means

1. Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
X	19.417[b]	.670	32.740	18.053	20.780
Y	21.115[b]	.621	32.267	19.850	22.380
Z	20.701[b]	.658	33.042	19.363	22.039

a. Dependent Variable: PD_SS3_Total.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS3_Total = 18.79, Baseline_sft = 33.98.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound
Y	X	1.698	.915	32.556	.140	-.446
Z	X	1.285	.941	32.786	.330	-.920

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	32.681	1.828	.177

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS3_Total.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
X	1	19.172[b]	.925	117.614	17.340	21.005
	2	18.928[b]	.922	119.013	17.103	20.754
	3	20.149[b]	.901	113.686	18.364	21.934
Y	1	20.637[b]	.834	112.809	18.985	22.289
	2	21.240[b]	.870	120.885	19.519	22.962
	3	21.467[b]	.834	113.184	19.814	23.120
Z	1	20.041[b]	.878	112.690	18.302	21.780
	2	21.480[b]	.896	117.567	19.705	23.255
	3	20.582[b]	.921	120.683	18.759	22.406

a. Dependent Variable: PD_SS3_Total.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS3_Total = 18.79, Baseline_sft = 33.98.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	1.465	1.245	115.598	.425		
	Z	X	.869	1.274	115.440	.747		
2	Y	X	2.312	1.266	120.451	.136		
	Z	X	2.552	1.286	117.886	.097		
3	Y	X	1.318	1.229	113.714	.490		
	Z	X	.434	1.289	116.844	.931		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	114.359	.693	.502
2	2	119.319	2.398	.095
3	2	116.186	.608	.546

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS3_Total.

Mixed Model Analysis – Serial Subtraction Errors (3's)

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_SS3_Errors	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	501.812
Akaike's Information Criterion (AIC)	505.812
Hurvich and Tsai's Criterion (AICC)	505.890
Bozdogan's Criterion (CAIC)	513.912
Schwarz's Bayesian Criterion (BIC)	511.912

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_SS3_Errors.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	56.590	27.427	.000
Treatment	2	49.603	3.253	.047
Rep	2	103.080	.313	.732
Treatment * Rep	4	102.319	.285	.887
Baseline_SS3_Errors	1	152.202	1.332	.250
Baseline_sft	1	54.715	1.257	.267

a. Dependent Variable: PD_SS3_Errors.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	1.031698	.149006
Intercept [subject = Participant]	Variance	.081791	.107688

a. Dependent Variable: PD_SS3_Errors.

Estimated Marginal Means

1. Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
X	1.171[b]	.156	50.379	.857	1.485
Y	1.137[b]	.144	48.754	.847	1.427
Z	.678[b]	.153	49.486	.370	.986

a. Dependent Variable: PD_SS3_Errors.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS3_Errors = .90, Baseline_sft = 33.98.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[b]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	Lower Bound	
Y	X	-.034	.213	49.759	.984	-.525	
Z	X	-.493	.219	50.017	.057	-.999	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	49.601	3.253	.047

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS3_Errors.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval	
					Lower Bound	Upper Bound
X	1	1.132[b]	.257	154.467	.624	1.641
	2	1.154[b]	.256	154.608	.648	1.660
	3	1.225[b]	.251	154.244	.730	1.720
Y	1	1.208[b]	.231	154.056	.752	1.663
	2	1.081[b]	.243	154.868	.601	1.561
	3	1.122[b]	.231	154.351	.666	1.577
Z	1	.912[b]	.245	154.141	.428	1.395
	2	.619[b]	.249	154.483	.127	1.110
	3	.503[b]	.257	154.320	-.006	1.011

a. Dependent Variable: PD_SS3_Errors.

b. Covariates appearing in the model are evaluated at the following values:

Baseline_SS3_Errors = .90, Baseline_sft = 33.98.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	.075	.346	154.229	.970		
	Z	X	-.220	.353	154.270	.782		
2	Y	X	-.073	.354	154.739	.973		
	Z	X	-.536	.357	154.512	.254		
3	Y	X	-.103	.340	154.229	.943		
	Z	X	-.723	.357	154.317	.088		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	154.179	.409	.665
2	2	154.614	1.353	.262
3	2	154.295	2.409	.093

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS3_Errors.

Mixed Model Analysis – Serial Subtractions Total (7s)

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_SS7_Total	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	779.706
Akaike's Information Criterion (AIC)	783.706
Hurvich and Tsai's Criterion (AICC)	783.784
Bozdogan's Criterion (CAIC)	791.818
Schwarz's Bayesian Criterion (BIC)	789.818

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_SS7_Total.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	59.975	14.617	.000
Treatment	2	46.262	.709	.497
Rep	2	99.339	5.239	.007
Treatment * Rep	4	99.402	.229	.922
Baseline_SS7_Total	1	65.534	299.136	.000
Baseline_sft	1	55.800	.916	.343

a. Dependent Variable: PD_SS7_Total.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	6.034528	.885343
Intercept [subject = Participant]	Variance	.254714	.622127

a. Dependent Variable: PD_SS7_Total.

Estimated Marginal Means

1. Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
X	13.256[b]	.365	45.277	12.522	13.991
Y	13.764[b]	.340	47.986	13.081	14.447
Z	13.790[b]	.343	45.199	13.098	14.482

a. Dependent Variable: PD_SS7_Total.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS7_Total = 12.92, Baseline_sft = 32.96.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	
Y	X	.508	.499	46.612	.530	-.646	
Z	X	.534	.501	45.338	.500	-.626	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	46.261	.709	.497

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS7_Total.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
X	1	12.516[b]	.610	156.089	11.312	13.721
	2	13.709[b]	.608	156.217	12.508	14.910
	3	13.543[b]	.609	156.088	12.339	14.747
Y	1	12.792[b]	.561	156.288	11.684	13.901
	2	14.119[b]	.576	156.499	12.981	15.256
	3	14.380[b]	.563	156.315	13.268	15.493
Z	1	12.957[b]	.562	156.033	11.847	14.068
	2	13.776[b]	.594	156.439	12.602	14.949
	3	14.637[b]	.561	156.184	13.529	15.745

a. Dependent Variable: PD_SS7_Total.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS7_Total = 12.92, Baseline_sft = 32.96.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	.276	.830	156.127	.932		
	Z	X	.441	.828	156.134	.836		
2	Y	X	.410	.837	156.326	.860		
	Z	X	.067	.851	156.267	.996		
3	Y	X	.837	.830	156.169	.530		
	Z	X	1.094	.829	156.073	.342		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	156.121	.144	.866
2	2	156.364	.141	.869
3	2	156.129	.932	.396

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS7_Total.

Mixed Model Analysis – Serial Subtractions Errors (7s)

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_SS7_Errors	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	502.902
Akaike's Information Criterion (AIC)	506.902
Hurvich and Tsai's Criterion (AICC)	506.980
Bozdogan's Criterion (CAIC)	515.001
Schwarz's Bayesian Criterion (BIC)	513.001

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_SS7_Errors.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	66.441	21.449	.000
Treatment	2	50.714	4.266	.019
Rep	2	102.434	.127	.881
Treatment * Rep	4	103.019	1.807	.133
Baseline_SS7_Errors	1	148.893	15.523	.000
Baseline_sft	1	56.280	.042	.839

a. Dependent Variable: PD_SS7_Errors.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	1.072363	.155074
Intercept [subject = Participant]	Variance	.045404	.105286

a. Dependent Variable: PD_SS7_Errors.

Estimated Marginal Means

1. Treatment

Estimates[a]

				95% Confidence Interval	
Treatment	Mean	Std. Error	df	Lower Bound	Upper Bound
X	1.298[b]	.156	51.460	.984	1.611
Y	1.117[b]	.144	51.032	.828	1.406
Z	.700[b]	.145	48.804	.409	.990

a. Dependent Variable: PD_SS7_Errors.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS7_Errors = 1.03, Baseline_sft = 33.04.

Pairwise Comparisons[a]

						95% Confidence Interval for Difference[c]	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[c]	Lower Bound	
Y	X	-.181	.214	52.187	.642	-.673	
Z	X	-.598*	.213	49.802	.014	-1.089	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	50.730	4.266	.019

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS7_Errors.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval	
					Lower Bound	Upper Bound
X	1	1.135[b]	.259	155.480	.625	1.646
	2	1.306[b]	.264	155.509	.784	1.828
	3	1.452[b]	.258	155.398	.943	1.962
Y	1	1.574[b]	.237	155.451	1.107	2.042
	2	.826[b]	.245	155.281	.342	1.310
	3	.951[b]	.237	155.438	.483	1.419
Z	1	.576[b]	.236	155.296	.109	1.043
	2	.892[b]	.252	155.792	.394	1.390
	3	.631[b]	.237	155.348	.163	1.099

a. Dependent Variable: PD_SS7_Errors.

b. Covariates appearing in the model are evaluated at the following values: Baseline_SS7_Errors = 1.03, Baseline_sft = 33.04.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[c]		
1	Y	X	.439	.351	155.397	.381		
	Z	X	-.559	.351	155.319	.213		
2	Y	X	-.480	.360	155.493	.335		
	Z	X	-.414	.366	155.563	.452		
3	Y	X	-.501	.351	155.450	.286		
	Z	X	-.821*	.352	155.333	.041		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	155.374	4.479	.013
2	2	155.538	1.019	.363
3	2	155.395	2.743	.067

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_SS7_Errors.

Mixed Model Analysis – RVIP Correct

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_RVIP_Correct	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	1288.552
Akaike's Information Criterion (AIC)	1292.552
Hurvich and Tsai's Criterion (AICC)	1292.635
Bozdogan's Criterion (CAIC)	1300.533
Schwarz's Bayesian Criterion (BIC)	1298.533

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_RVIP_Correct.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	112.714	36.248	.000
Treatment	2	45.165	1.239	.299
Rep	2	90.045	1.029	.361
Treatment * Rep	4	90.345	.486	.746
Baseline_RVIP_Correct	1	133.054	34.160	.000
Baseline_sft	1	44.477	1.711	.198

a. Dependent Variable: PD_RVIP_Correct.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	195.151245	30.189487
Intercept [subject = Participant]	Variance	105.003016	40.761349

a. Dependent Variable: PD_RVIP_Correct.

Estimated Marginal Means

1. Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
X	70.371[b]	3.091	44.840	64.144	76.598
Y	76.759[b]	2.940	44.350	70.836	82.682
Z	75.601[b]	3.127	45.890	69.307	81.895

a. Dependent Variable: PD_RVIP_Correct.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_Correct = 75.791, Baseline_sft = 33.73.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	
Y	X	6.388	4.264	44.620	.262	-3.480	
Z	X	5.230	4.429	45.475	.428	-5.012	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	45.167	1.239	.299

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_Correct.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
X	1	69.704[b]	4.244	122.012	61.303	78.105
	2	70.012[b]	4.084	116.894	61.925	78.100
	3	71.396[b]	4.314	124.575	62.858	79.934
Y	1	77.163[b]	4.140	128.190	68.972	85.355
	2	79.252[b]	3.966	119.180	71.399	87.106
	3	73.860[b]	3.798	113.541	66.336	81.385
Z	1	75.488[b]	4.295	125.200	66.988	83.988
	2	79.530[b]	4.094	116.155	71.421	87.638
	3	71.785[b]	4.299	124.503	63.277	80.294

a. Dependent Variable: PD_RVIP_Correct.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_Correct = 75.791, Baseline_sft = 33.73.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	7.459	5.904	123.972	.374		
	Z	X	5.784	6.087	122.880	.569		
2	Y	X	9.240	5.703	117.750	.204		
	Z	X	9.517	5.805	115.816	.197		
3	Y	X	2.464	5.709	120.193	.889		
	Z	X	.389	6.111	124.122	.997		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	124.566	.865	.424
2	2	117.099	1.762	.176
3	2	121.176	.112	.894

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_Correct.

Mixed Model Analysis – RVIP Correct RT

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_RVIP_CorrectRT	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	1626.838
Akaike's Information Criterion (AIC)	1630.838
Hurvich and Tsai's Criterion (AICC)	1630.922
Bozdogan's Criterion (CAIC)	1638.819
Schwarz's Bayesian Criterion (BIC)	1636.819

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_RVIP_CorrectRT.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	139.021	137.511	.000
Treatment	2	45.634	.700	.502
Rep	2	90.780	1.006	.370
Treatment * Rep	4	90.112	.763	.552
Baseline_RVIP_CorrectRT	1	144.116	7.293	.008
Baseline_sft	1	44.449	.092	.763

a. Dependent Variable: PD_RVIP_CorrectRT.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	2049.494506	315.913912
Intercept [subject = Participant]	Variance	785.516270	360.690579

a. Dependent Variable: PD_RVIP_CorrectRT.

Estimated Marginal Means

1. Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
X	487.962[b]	9.116	45.000	469.602	506.323
Y	473.129[b]	8.670	44.879	455.666	490.592
Z	480.820[b]	9.262	46.503	462.181	499.458

a. Dependent Variable: PD_RVIP_CorrectRT.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_CorrectRT = 483.2914, Baseline_sft = 33.73.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	
Y	X	-14.833	12.552	44.892	.428	-43.873	
Z	X	-7.142	13.083	45.804	.830	-37.391	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	45.636	.700	.502

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_CorrectRT.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
X	1	497.467[b]	12.907	130.449	471.934	523.001
	2	492.704[b]	12.584	126.912	467.803	517.604
	3	473.715[b]	13.213	133.235	447.581	499.849
Y	1	475.321[b]	12.792	135.163	450.023	500.619
	2	465.043[b]	12.192	129.442	440.922	489.164
	3	479.024[b]	11.638	124.033	455.990	502.059
Z	1	491.576[b]	13.257	133.183	465.355	517.797
	2	472.503[b]	12.612	125.918	447.545	497.461
	3	478.379[b]	13.282	132.607	452.107	504.652

a. Dependent Variable: PD_RVIP_CorrectRT.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_CorrectRT = 483.2914, Baseline_sft = 33.73.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	-22.147	18.145	133.218	.398		
	Z	X	-5.891	18.526	131.492	.938		
2	Y	X	-27.661	17.455	128.157	.218		
	Z	X	-20.200	17.921	125.894	.455		
3	Y	X	5.309	17.592	129.621	.944		
	Z	X	4.664	18.797	132.333	.962		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	133.010	.798	.452
2	2	127.247	1.327	.269
3	2	130.183	.051	.950

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_CorrectRT.

Mixed Model Analysis – RVIP False Alarms

Model Dimension[a]

		Number of Levels	Covariance Structure	Number of Parameters		
Fixed Effects	Intercept	1		1		
	Treatment	3		2		
	Rep	3		2		
	Treatment * Rep	9		4		
	Baseline_RVIP_Fas	1		1		
	Baseline_sft	1		1		
Random Effects	Intercept[b]	1	Variance Components	1		
Repeated Effects	Rep	3	Identity	1		
Total		22		13		

Information Criteria[a]

-2 Restricted Log Likelihood	361.244
Akaike's Information Criterion (AIC)	365.244
Hurvich and Tsai's Criterion (AICC)	365.327
Bozdogan's Criterion (CAIC)	373.225
Schwarz's Bayesian Criterion (BIC)	371.225

The information criteria are displayed in smaller-is-better form.[a]

a. Dependent Variable: PD_RVIP_FAs.

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	49.780	7.189	.010
Treatment	2	44.699	1.017	.370
Rep	2	92.995	.254	.776
Treatment * Rep	4	92.357	.195	.940
Baseline_RVIP_Fas	1	134.049	16.278	.000
Baseline_sft	1	42.305	.025	.876

a. Dependent Variable: PD_RVIP_FAs.

Covariance Parameters

Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Repeated Measures	Variance	.474395	.072782
Intercept [subject = Participant]	Variance	.044980	.056286

a. Dependent Variable: PD_RVIP_FAs.

Estimated Marginal Means

1. Treatment

Estimates[a]

Treatment	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
X	.559[b]	.110	45.055	.338	.780
Y	.345[b]	.103	43.038	.136	.553
Z	.451[b]	.111	45.384	.228	.675

a. Dependent Variable: PD_RVIP_FAs.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_Fas = .40, Baseline_sft = 33.73.

Pairwise Comparisons[a]

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	
Y	X	-.214	.151	43.938	.297	-.563	
Z	X	-.107	.158	45.506	.750	-.473	

Univariate Tests[a]

Numerator df	Denominator df	F	Sig.
2	44.705	1.017	.370

The F tests the effect of Treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_FAs.

2. Treatment * Rep

Estimates[a]

Treatment	Rep	Mean	Std. Error	df	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
X	1	.598[b]	.176	145.098	.250	.946
	2	.484[b]	.171	144.234	.147	.821
	3	.595[b]	.180	145.305	.238	.951
Y	1	.435[b]	.175	145.727	.089	.781
	2	.301[b]	.165	144.983	-.026	.628
	3	.298[b]	.159	143.852	-.016	.612
Z	1	.488[b]	.182	145.414	.129	.847
	2	.515[b]	.171	143.998	.178	.853
	3	.351[b]	.181	144.740	-.007	.709

a. Dependent Variable: PD_RVIP_FAs.

b. Covariates appearing in the model are evaluated at the following values: Baseline_RVIP_Fas = .40, Baseline_sft = 33.73.

Pairwise Comparisons[a]

Rep	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.[b]		
1	Y	X	-.163	.247	145.403	.760		
	Z	X	-.110	.255	145.204	.889		
2	Y	X	-.183	.237	144.508	.687		
	Z	X	.032	.242	143.715	.989		
3	Y	X	-.297	.241	144.709	.392		
	Z	X	-.244	.257	144.615	.569		

Univariate Tests[a]

Rep	Numerator df	Denominator df	F	Sig.
1	2	145.412	.225	.798
2	2	144.203	.483	.618
3	2	144.706	.818	.443

Each F tests the simple effects of Treatment within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.[a]

a. Dependent Variable: PD_RVIP_FAs.